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THE ENDOCRINE CONTROL OF AMPHIBIAN METAMORPHOSIS

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IN discussing the more recent experimental work upon the endocrine ~~glands in~~ their relation to amphibian metamorphosis, we cannot leave wholly out of account the earlier work that forms a basis for it; but the great volume of investigation in this field necessitates a limitation in an article of this character, more justified because of the fact that the writer published a survey article of this type in 1929 (Allen, 1929). For this reason the details of our study will concern chiefly such papers as appeared since that date, yet we must develop the basic principles of these earlier studies extending back to the year 1912 in order to lay our foundations.

The pioneer work of Gudernatsch (1912), in which he demonstrated the fact that thyroid gland material fed to amphibians accelerates metamorphosis, was followed by studies by Allen (1916) and Hoskins, E. R. & M. M. (1917), in which the converse principle was demonstrated, namely that removal of the thyroid rudiment prevents metamorphosis.

The importance of iodine in the physiology of the thyroid gland has been recognized for many years, but the first experiments to test whether elemental iodine could induce amphibian metamorphosis were negative. Morse (1914) used a variety of iodine compounds such as elemental iodine, potassium iodide, iodobenzoic acid, and iodoxy-benzoic acid. In all these cases he immersed tadpoles in solutions of the substances named above but in no instance was any tendency toward metamorphosis observed. He even followed the method of feeding iodine combined with starch, but without inducing metamorphosis. Swingle (1919), using a similar method, was the first to show that elemental iodine used alone can induce metamorphosis not only in normal amphibian larvae, but even in those from which the thyroid gland had been removed. Because of the well-known fact that the thyroid gland maintains its activity only under the stimulus of the anterior lobe of the hypophysis, Allen (1919) fed iodine to tadpoles from which the hypophysis had been removed and also to those from which both hypophysis and thyroid had been removed. In all these cases the iodine induced metamorphosis, demonstrating clearly that these glands, so essential for the utilization of the minute quantities of iodine normally present in food and water, are not essential in the utilization of iodine when present in large amounts. Swingle (1919) gave quantitative data upon this point by demonstrating that normal *Rana* tadpoles can be induced to meta-

morphose by immersion in a solution of 0.000003833 per cent of elemental iodine which was the weakest in his experiments, but double this concentration did not induce metamorphosis in tadpoles that had been thyroidectomized.

For some time doubts were raised as to whether elemental iodine could induce metamorphosis of urodeles as well as anurans. Uhlenhuth (1921) failed to induce metamorphosis in the salamander *Ambystoma* by the use of elemental iodine. From these observations he drew the conclusion that the manufacture and storage of the hormone in the thyroid gland does not affect the animals, the real difficulty being explainable on the basis of a failure to release the hormone from the thyroid gland. He also concluded that urodeles are induced to metamorphose only by the thyroid hormone and not by elemental iodine. But Huxley & Hogben (1922) caused *Triton* larvae to metamorphose by immersing them in dilute solutions of iodine. These investigators were not so fortunate, however, in inducing metamorphosis of the axolotl. Huxley (1920) performed this line of work with axolotls by feeding iodine to them and keeping them in iodine solutions. At most, however, he produced only slight effects comparable with early stages of metamorphosis. On the other hand, Hirschler (1922), Blacher & Belkin (1927), and Zawadowsky *et al.* (1928) induced metamorphosis in this form by intraperitoneal implantation of iodine crystals. Not only can normal axolotls be induced to metamorphose as a result of iodine administration, but Swingle (1922 *c*) showed that injection with strong doses of di-iodotyrosine and iodized serum globulin induced metamorphosis quite readily. More strikingly still, Ingram (1928 *b*), injecting a solution of elemental iodine, induced metamorphosis of Colorado axolotls that had been thyroidectomized and hypophysectomized.

Lynn & Brambel (1935) reared tadpoles of *Rana sylvatica* in distilled water, feeding them with rolled oats deficient in iodine. Although they lived 116 days, they showed no signs of hindlimb growth, but other groups reared in distilled water with iodine did show hindlimb growth. This experiment is significant in showing that metamorphosis does not occur in the practical absence of iodine.

A whole series of experiments discussed in my earlier article show that the effectiveness of iodine in inducing metamorphosis of tadpoles is largely dependent upon the nature of its combination with other elements. Swingle (1919) found that iodine is more potent in this regard than potassium iodide and iodoform. Potassium iodate was ingested by the tadpoles but, so far as his observations went, it did not cause metamorphosis. This he attributed to the inability to release the iodine by breaking down the molecule.

The potency of diiodotyrosine in inducing metamorphosis has been shown by quite a number of workers: Morse (1914), Abderhalden & Schiffmann (1922), Abelin (1921), Swingle (1922 *b*) and Romeis (1922). Several of these investigators showed that tyrosine alone has no such effect. While Swingle (1922 *c*) demonstrated that dibromotyrosine is equally ineffective, Kendall (1919) found that acetylated thyroxin, while having lost its property of influencing basal metabolism of mammals, retains its full power to induce metamorphosis in amphibians. These findings were completely substantiated by Swingle *et al.* (1924). The obvious conclusion from all of this is that iodine is the effective agent and that its effectiveness varies accord-

ing to the character of the chemical combination in which it occurs. This has been brought out most clearly in a series of unpublished experiments carried out by Allen Lein, one of my students, who placed tadpoles of *R. aurora draytoni* in solutions of potassium iodide, 3-5-diiodotyrosine, and thyroxin made up in such percentage that they contained comparable amounts of iodine. It was shown that thyroxin is by far the most potent, considering its iodine content, di-iodotyrosine being next, and potassium iodide last. According to his experiments iodine in the thyroxin molecule has over 300 times the potency in inducing amphibian metamorphosis than has an equal amount as contained in the di-iodotyrosine molecule. There has been some evidence adduced to show that thyroxin in thyroglobulin combination as it occurs in the thyroid gland is more potent than when used in a purified form. In any case, the qualitative influence of thyroxin, of various iodine combinations, and of elemental iodine itself on amphibian metamorphosis gives the characteristic thyroid picture.

Gunthorp (1932) investigated the relative potency of thyroid preparations from different types of animals in their effect upon amphibian metamorphosis when fed quantitatively. While he found a general correspondence between the iodine content and the effect produced, human and cat thyroids showed an effect decidedly greater than the iodine content would seem to warrant. These facts may probably find their explanation in differences in the proportion of iodine, di-iodotyrosine and thyroxin in the thyroid glands of different species, the potency being largely dependent upon the amount of thyroxin. There may be also differences of potency due to the protein combinations. In this connection it was shown by Hoffman & Gudernatsch (1933) that certain amino acids, when combined with di-iodotyrosine, contribute to the influence which that substance exerts upon the metamorphosis of tadpoles. For instance, when they mixed equal amounts of solutions of tyrosine or tryptophane and di-iodotyrosine, the combination representing the di-iodotyrosine diluted to one-half exerts as much effect as the full strength di-iodotyrosine solution alone. This principle was found to apply to several amino acids which they called "differentiation" acids.

There has been some effort to work out a tadpole test to determine the toxicity of goitre. This has its defects in the fact that the amount of stored secretion may be no index of the secretory activity of the gland and that iodine medication may seriously complicate the picture. Lenhart (1915) found that goitre material from both simple and exophthalmic types induces metamorphosis but not as readily as does material from normal thyroid glands. Graham (1916) similarly found that normal thyroid gland is more effective than that from adenomatous goitre. There has been some disagreement as to whether the influence of goitre material upon amphibian metamorphosis is strictly proportional to the iodine content. Graham (1916), together with Abderhalden & Schiffmann (1922), hold that this is the case, while Abelin (1927) and Cooksey & Rosenblatt (1928) concluded from their experiments that the influence of these pathological thyroid glands upon amphibian metamorphosis is not strictly proportional to their iodine content. We can readily understand why this latter conclusion could be true.

One might naturally inquire whether thyroid substance from warm-blooded animals would produce heat in cold-blooded forms. Gayda (1922) fed and injected thyroid preparations from mammals into frogs but found that there was very little heat production induced. This was in spite of greatly increased oxygen consumption demonstrated by Huxley's (1925) experiments in which he caused *R. temporaria* larvae to show a 100 per cent increase of oxygen intake as a result of exposure to fairly strong thyroid solutions. Kendall (1919) and later Swingle *et al.* (1924) demonstrated that acetylated thyroxin had lost its influence on basal metabolism of mammals, but had suffered no change in its power to induce amphibian metamorphosis. The converse situation is found to apply in the case of dinitrophenol which has been much used of late to stimulate metabolism with the well-known medical application of reducing obesity. Administration of this drug to tadpoles has been shown by Cutting & Tainter (1933) to have no tendency to stimulate metamorphosis. This emphasizes the conclusion that metabolism does not necessarily involve the process of metamorphosis. While the thyroid function entails both of these processes in the amphibians, dinitrophenol involves only metabolism. This discovery is in line with the effects of acetylated thyroxin as described above. Among the studies of metabolic activity during metamorphosis we may mention the work of Helff (1926 *b*) who made over 500 Winkler oxygen-consumption tests on *R. pipiens* tadpoles that were stimulated by thyroid preparations and by diiodotyrosine. In the earliest stage studied prior to the changes of metamorphosis, this treatment caused no increase of oxygen consumption, but as development proceeded there was observed a "progressive increase in oxygen consumption per gram weight until in later stages it reached a level 79 per cent above that of the earliest stage tested". He found that di-iodotyrosine proved to be very effective in inducing metamorphosis, but that the changes are of a type somewhat different from the normal. During the later stages tadpoles undergo a reduction of weight amounting to 57 per cent. In the case both of thyroxin and diiodotyrosine there is a marked increase in oxygen consumption, but Helff considers that this is secondary and not causative of metamorphosis. Tchepovetsky (1934) found that thyroxin with high temperature induces metamorphosis in the axolotl in direct proportion to the amount or degree up to a certain point. High temperature alone, however, is not effective.

Huxley (1929) showed that tadpoles of *R. temporaria* exposed to a filtered suspension of thyroid powder undergo the usual metamorphosis except when kept at a temperature below 5° C., in which case the initial stimulus halts before the completion of metamorphosis, the tadpoles remaining for a long time in the "half-and-half" condition. While this may be partially explained as a result of a compensatory reduction of the animal's own thyroid gland, Huxley concludes that some of the influence of the thyroid hormone administered "is used up in counteracting the effects of low temperature instead of in producing metamorphosis".

It is well to consider the ability of the organism to continue responding to a stimulus that has once been given but subsequently withdrawn. A very clever experiment to this end was carried out by M. M. Hoskins (1922), who transplanted thyroid glands to the tails of tadpoles. When these attained their full size she cut

off the tails together with the transplanted gland and found that the tadpoles usually continued through to complete metamorphosis in spite of the removal of the stimulus. We may ask whether this is due to the continuance of processes set in motion or to retention of thyroxin in the tissues.

Kahn (1924) induced metamorphosis in tadpoles by thyroid feeding and then fed their tissues to other tadpoles without influencing the latter toward metamorphosis. On the other hand, Abderhalden & Wertheimer (1928) tested liver, muscle, blood corpuscles, etc., that had been soaked in thyroxin and found there was considerable absorption and retention of thyroxin. This was especially true of muscle tissue which holds the hormone so tenaciously that it cannot be soaked, compressed or boiled out of the tissues. Zawadowsky & Novikov (1926) investigated the relative role of blood serum and erythrocytes in retaining thyroxin, concluding that it is held in the serum but not in the corpuscles.

Quite recently Binswanger (1936) showed that tadpoles left for 24 hours in a solution of thyroxin and then placed in water wholly free from it continued their development unless kept at a low temperature of 4–6° C., in which case they failed to develop. He concluded that the animals have the capacity to store reserves of iodine.

A number of workers have studied the age at which tadpoles show an ability to respond to thyroid stimulus by changes of metamorphosis. Among these are Romeis (1924), Jarisch (1920), and Champy (1922). As Romeis points out, the specific effect of thyroid extracts upon anurans appears only at a stage after the operculum covers the external gills.

These findings have been verified by Allen in unpublished work to test the influence of thyroxin solutions ranging to a high degree of concentration, upon the rate of development in early stages of *R. aurora* and *Bufo holophilus*. During the segmentation, gastrulation, neural fold and succeeding stages, up to a stage differing in different species, thyroxin exerts no stimulating influence whatsoever upon the rate of development, after which time its importance is paramount and practically no further development can take place in its absence. Sklower (1925), Mayerowna (1922) Hirschlerowa (1928), Clements (1932), Aleschin (1936), and Brink (1936) have all studied the cytological changes in the thyroid gland of anurans that occur during metamorphosis, while Uhlenhuth (1927), Hirsch (1928), and Grant (1931 a) have followed these processes in the urodeles. There is general unanimity in the conclusion that there are clear evidences of thyroid activity near the beginning of active metamorphosis. First there has been an accumulation of colloid during the early development of the hindlegs, followed by active colloid discharge resulting in the rapid body changes of metamorphosis such as shrinkage of gills, intestine and tail, and growth of forelimbs.

These changes involve erosion and liquefaction of colloid masses within the follicles and the appearance of more or less fluid colloid droplets within the follicular cells. While these first appear within the inner apical portions of the cells, they pass toward the basal portions, increasing in size as they do so. There is some difference of opinion as to their mode of discharge, whether from the bases of the cells or

intracellular. Upon the completion of metamorphosis the follicles again contain solid rounded masses of colloid and intracellular droplets have become scarce. During the height of activity the follicular cells become columnar to again flatten out when the active discharge ceases.

Grab (1933) showed that while colloid is not itself the active hormone, at least 95 per cent of the thyroxin and di-iodotyrosine are contained in it.

Aleschin (1936) found that administration of thyroid tablets to larvae of *Rana temporaria* increases the activity of their own thyroid glands during the period of metamorphosis and shortly prior to that process; but administration after metamorphosis lessens thyroid activity. We may be permitted to suggest that this might be due to an action of the thyroid hormone in hastening the development of the anterior lobe of the hypophysis. Administration of thyroid hormone to hypophysectomized tadpoles would throw some light upon this problem.

Brink (1936) finds an interesting difference in colloid behaviour between *Bufo* and *Arthroleptella*. In the former there is an accumulation of colloid in the follicles prior to metamorphosis while in the latter this does not take place, colloid secretion and discharge occurring simultaneously. Contrary to Aleschin (1936) he finds that in these forms thyroid administration suppresses the normal differentiation and activity of the thyroid gland of anuran larvae so that during and after metamorphosis it remains in a stage of development like that of a larva in which no trace of approaching metamorphosis has appeared. This contradiction can be resolved only by taking the anterior lobe of the hypophysis into account as we have suggested, and by having a clearer understanding of the significance of the morphological features observed.

One may inquire further as to the details of the relation of the development and growth of the thyroid gland to the changes of metamorphosis in the tadpole. Etkin (1930, 1935 a) has given us a very painstaking analysis of this question by making counts of mitotic figures in the cells of the thyroid gland and determinations of the colloid content, using *Rana pipiens* as the object of his study. He finds that there is very little development of the thyroid gland during the early stages of "pro-metamorphosis" but that increase in cell number and colloid content becomes very striking between the time when the hindlegs begin active growth and the beginning of resorption of the tail. From this period of stormy activity of the thyroid gland, "metamorphic climax", it assumes a diminished growth as shown by the lessened proportion of cell number and colloid content as compared with the cube of body length. During this stage of greatest activity the cells of the follicular epithelium are mostly columnar in form. This work of Etkin's substantiates the general findings of a paper by the writer (Allen, 1919) upon *Bufo americanum*.

Champy (1922) has made an exhaustive study of the effect of the thyroid hormone upon cell structure, mitosis and cell degeneration in *Rana temporaria*. In the case of the intestine, thyroid administration caused both cell degeneration and at the same time mitosis, the two processes resulting in a complete reorganization of the organ.

He found that the gonads not only fail to develop in response to thyroid stimulus as Swingle (1918 b) had noted, but his more intensive dosage actually caused

degeneration of the germ cells and regression of the gonads, which was more pronounced in the female than in the male.

Champy finds that thyroid stimulation is selective in the sense of stimulating certain regions and leaving others unaffected. The stimulus as measured by counting mitoses is applied to the various kinds of tissue found in the regions in question. The result of this local increase of activity combined with the complete reorganization of the alimentary tract brings about profound inanition of the non-stimulated parts.

He advances the view that the parts affected have certain chemical, cytological, or physical characteristics that make them responsive to the thyroid hormone and emphasizes that these thyroid stimulated structures are in detail the organs characteristic of life on land.

Etkin (1932) explains the orderly succession of steps in metamorphosis as being due to differences in the threshold of response of the different tissues, those like the hindlegs that respond during the earlier "prometamorphic period" having a low threshold of response, while other processes like the resorption of the tail, taking place during the "metamorphic crisis", have a higher threshold of response. He lists the different events in the metamorphosis of *R. catesbiana* and *R. clamitans* in the following order:

(1) Hindlegs reach 10 mm.; differentiation of adult regions completed; (2) terminal part of intestine shortened to level of trunk; (3) skin window for forelegs distinctly visible; (4) emergence of both forelegs; (5) loss of chitinous beak; (6) eyelids developed, eyes enlarging; (7) flakes of shed skin appearing; (8) beginning of tail resorption; (9) breathing through nostrils; (10) widening of mouth, etc. Other forms of amphibians would show different patterns of response to the thyroid influence according to the genetic factors that must certainly condition them.

Romeis (1923 b) followed the method of immersing tadpoles in solutions of iodothylin, iodoglobulin, diiodotyrosine, and thyroxin. The last named proved to be very much more powerful than the others in inducing metamorphosis. The animals were exposed to different concentration of the hormone for different periods of time and were afterwards placed in tap water. The character and degree of development induced depends upon the concentration of the solution, the length of exposure to it and the stage of development of the tadpole; also, in some degree, to individual variation in susceptibility. Romeis caused some acceleration of development in tadpoles kept in solutions of thyroxin as dilute as 1 to 1,000,000,000 during the entire period of their larval life. This solution effected only the development of the hindlimbs, one of the most susceptible of all characters of the tadpole.

Kosmin & Resnitschenko (1927) worked in similar manner, concluding that the rapidity of response increases with the age of the tadpoles.

Blacher (1928) used thyroidin, the powdered gland substance, in similar experiments in which different concentrations in tap water were used, the tadpoles being immersed for a uniform period of 3 days. He determined the threshold of response of the various features of the tadpoles, noting the thyroid concentration necessary

to induce changes of metamorphosis. These showed the following order of responsiveness:

(1) Intestine length, (2) tail length, (3) trunk length, (4) loss of horny jaws, and (5) eruption of forelimbs. These degrees of susceptibility were not due to the accessibility of the structures but to their specific responsiveness to the degree of stimulus.

Allen (1932 *a*) dealt with this problem in somewhat different fashion by using different concentrations of thyroxin ranging from 1 to 200,000, up to 1 to 1,000,000,000. During the time period of the experiment (10 days) no response of any kind was attained in concentrations less than 1 to 200,000,000. It was found that certain features like hindleg length and length of the alimentary tract showed a ready response to low concentrations, while other features responded much more slowly to these concentrations, but readily enough at higher ones. "Our experiments show that this 'threshold of response' is really a question of 'time of response'; structures that do not develop as quickly as others in weak solutions will respond in these same solutions after longer exposure to them." In this work no stimulus was produced in stages prior to the first appearance of the hindlimb buds.

Etkin (1935 *a*) substantiated his above listed order of development by using different thyroxin dilutions, developing the significant point that a normal pattern of development can be brought about only by the use of gradually increasing concentrations of thyroxin corresponding with the gradual development of the thyroid gland. Huxley (1925) called attention to the fact that in the anuran the limbs respond to a low concentration of thyroid material, while the tail is affected only by a much stronger stimulus.

Danforth (1933) in discussing the influence of sex hormones upon birds has used the very apt phrase that a species or breed has developed the capacity for *using* a given hormone in a particular way, according to its own special pattern of response. This point of view seems to us worthy of very general application, and may be applied quite properly to the phenomena in question. To give only one illustration, the thyroid gland causes anuran larvae to lose the tail, but in urodeles it causes only the loss of the membranous portion.

Quite diverse features of growth and regeneration of the tail under the influence of thyroid powder added to the aquarium water have been shown by Speidel (1929) who worked out a series: toad, tree frog, wood frog, green frog, and bull frog, ranging from those forms in which tail resorption is induced by low concentrations to others, terminating in the bull frog, in which much higher concentrations are required to produce an effect. He found that limbs and gills respond in similar degree in the series. It is significant that in this series the greatest concentration is necessary to induce metamorphosis in those species, green frog and bull frog, which remain longest in the larval form.

It is quite possible for one to consider that perennibranchiates like *Necturus* are organisms whose distant ancestors underwent metamorphosis but have now completely lost the capacity to do so. With the example of the axolotl before us it is natural to suppose that thyroid treatment could be used to bring about meta-

morphosis in *Necturus* as well, but these efforts have met with very indifferent success. In 1918, the writer fed Armour's thyroid powder to adult *Necturus* without inducing any sign of metamorphosis and Swingle (1922 *a, b*) was no more successful. Apparently a slight effect was produced by Gutman (1926) using young *Necturus* 37 mm. in length. Simply immersing them in dilute solutions of thyroxin was ineffective but when adrenalin was added to it the *Necturus* showed considerable reduction of the gills, exophthalmos and change in the shape of the head. The rationale of this is not quite clear and studies along this line should be extended.

Swingle (1922 *a, b*) showed that the thyroid glands of both the Colorado axolotl *Ambystoma tigrinum* and of *Necturus* contain active thyroid hormones when he cut them into several pieces, each of which was capable of inducing metamorphosis in *Rana clamata* tadpoles when implanted into them. This might be explained on the grounds that these animals do not bring about a release of thyroxin by the influence of the thyrotropic hormone of the anterior lobe of the hypophysis but Swingle (1924) gave good evidence against this view by removing the thyroids from these animals, crushing them and injecting them intraperitoneally without inducing metamorphosis. Similarly, it was found by Charipper & Corey (1930) that transplants of the anterior lobe of the hypophysis of adult *Necturus* into larvae of *R. clamitans* accelerates their metamorphosis quite markedly. Thus, we see that both the thyroid gland and the anterior lobe of the hypophysis are potent in inducing metamorphosis in species that are capable of response.

Helff (1926 *a*) tested the factors responsible for the perforation of the skin to allow the protrusion of the fore limbs, finding that it is due to the influence of the degenerating gills. Transplantation of these structures under the skin of any part of the body produces this same result. Transplantation of pieces of the degenerating tail likewise causes perforation of the overlying skin. Transplantation of that skin normally perforated by the forelimbs to other parts of the body shows that that character is not a self-differentiating feature but is due to the influence of these degenerating tissues. Similarly, Helff (1931) found that the thickened yellow fibrous area of the tympanic membrane does not develop during metamorphosis unless it is brought into contact with the distal end of the developing columella. On the other hand, Helff (1930) disproved the older view that the urostyle causes the degeneration of the tail by pressure upon blood vessels at its base. His method was very simple, merely to remove the urostyle, in spite of which the tail degenerated, doubtless due to direct response to the thyroid hormone.

Regenerative processes are affected by thyroid treatment, according to the age of the tissue involved. Speidel (1929) set up a clever experiment to test this principle by cutting off a large portion of the tadpole tail and then cutting off the terminal half of the regenerated part, thus giving three types of tissue: the original tail substance about to undergo resorption, an older regenerated part, and the terminal portion still in the phase of proliferation. Treatment with thyroid hormone induces shrinkage of the original tail membrane first, then the oldest regenerated part, while the new terminal part for some time continues proliferative growth, only undergoing resorption when the tissues have entered into a more advanced stage of

differentiation. The regeneration of the hindlegs is retarded by administration prior to, during, or shortly after amputation, but when deferred to the stage of proliferation, this process is greatly stimulated. After causing marked growth in size, differentiation is finally induced after the tissues have reached a certain phase of development.

In much of the work upon metamorphosis of amphibians, the process of moulting has been studied as an essential part of the picture, and the first shedding of the skin has been used as one of the important criteria. Since the functioning of the thyroid gland is so completely under the control of the hypophysis, it is not surprising that Giusti & Houssay (1921, 1924), and Puente (1927) found in their work upon adult toads that total removal of the hypophysis completely prevents moulting, an effect due to the absence of the pars anterior. Adams & Richards (1929) inhibited the moult in *Triturus viridescens* adults by thyroidectomy, also by hypophysectomy. After such prevention the process was restored by introducing thyroid hormone or iodine, or by bringing about its release in the case of hypophysectomized animals. Adams (1932) has given a full account of the restoration of the moult by implantation of the thyroid gland into thyroidectomized animals and by transplantation of the hypophysis into hypophysectomized animals in which the moulting process had been previously halted.

The influence of the hypophysis is far-reaching, and much of our knowledge regarding its control of the other glands of internal secretion has been derived from experiments upon amphibians.

There is to-day no doubt regarding its influence upon the thyroid gland as demonstrated in many ways. One of the important methods of studying this problem is to inject emulsions or extracts of the hypophysis into normal or hypophysectomized tadpoles. Among the earliest attacks upon the problem from this angle was that by Smith & Smith (1923) in which they used an emulsion of the fresh pars anterior of the beef hypophysis that had been ground in sterile sand and then centrifuged. The effect of this treatment was quite clear in that the thyroid glands of hypophysectomized tadpoles were restored to normal function, and metamorphosis induced.

Spaul (1925-8) went a step farther in grinding the glandular material with weak acetic acid, thus producing an extract that he found to be fairly potent in causing metamorphosis of normal tadpoles. These hypophyseal preparations were never so active as similarly prepared extracts of the thyroid gland.

Smith (1926) prepared an extract of the anterior lobe of the beef hypophysis and tested its influence upon the metamorphosis of the Colorado axolotl, coming to the surprising conclusion that in this case it retards instead of stimulating metamorphosis. This extract was made by crushing the glands in such a way as to press out their juices and may have failed to secure the hormone. In any case, thyrotropic hormone of high potency has been made in recent years by various means. The use of commercial preparations of the hypophysis by Hogben (1923) in his experiments with the Mexican axolotl and in the earlier work of Spaul (1924), whereby they induced metamorphosis, are hardly convincing because of the demonstration by Smith & Cheney (1921) that certain at least of these commercial hypophyseal powders

contain considerable quantities of iodine. However, Spaul (1924, 1925 *a*) and Hogben as well (1923, 1927) clearly demonstrated the fact that the Mexican axolotl can be caused to metamorphose by using preparations of the anterior lobe of the hypophysis prepared in the laboratory from fresh gland material.

Spaul has done extensive pioneer work in producing extracts of the anterior lobe of the hypophysis. This has been greatly extended by the investigations of Collip, Evans, and Van Dyke, who have employed various techniques until we now have fairly pure thyrotropic hormone. Uhlenhuth & Schwartzbach (1928) were able to accelerate the metamorphosis of the Utah axolotl to the extent of 36 days by using Spaul's methods.

Spaul (1925 *b*, 1930) claims that the posterior lobe secretion counteracts influences making for metamorphosis. He finds that injections of posterior lobe extracts not only retard the development of normal tadpoles or axolotls but counteracts the influence of injected iodine and of extracts of the pars anterior of the hypophysis as well. In fact, he attributes the failure of Smith to induce metamorphosis of the Colorado axolotl to possible contamination with material of the posterior lobe, and this view was adopted by Uhlenhuth & Schwartzbach (1928). Spaul finds that the posterior lobe is not very potent in opposing powerful stimuli to metamorphosis and finds in certain cases that it produces an initial stimulus followed later by a retardation. It seems to the writer that we need further evidence upon the retarding influence of the posterior lobe of the hypophysis. Not only are we ignorant of the part of the posterior lobe involved but the very question of the existence of such a retarding influence calls for additional methods of experimentation. Transplantation of pure pars intermedia and pars nervosa tissues in a large series of experiments seems to be highly desirable.

Adler (1914) showed that destruction of the hypophysis prevents metamorphosis, and expressed the view that this effect is due to the dependence of the thyroid gland upon it. Better methods of removing the hypophysis at its first appearance were simultaneously worked out by Smith (1916 *a*) and Allen (1916) and have led to extensive subsequent research in which these relationships have been firmly established. All these experiments demonstrate that the thyroid gland does not function in the absence of the hypophysis, there being no accumulation of colloid. Allen (1925) taking hindlimb growth as a criterion of metamorphosis gave a series of data showing that their length is the same in tadpoles from which the thyroid gland has been removed, in those from which the hypophysis has been removed, and in those from which both have been removed. In all these cases, the hindlimbs reach a length characteristic of the species as shown in *Bufo americanum* and in *Rana pipiens*; in the former, they are much longer and more highly developed than in the latter. From this period onward they grow slightly with the further growth of the entire body of the tadpole but increase in proportion to it only slightly, if at all. At this period of development the thyroid gland, under the stimulus of the hypophysis, plays its part in the further development of the organism. It is thus seen that these endocrine glands take their place alongside of the genes, physiological gradient and organizer as an important part of the chain of influences that

control development. It would seem to be the last of these factors in point of time to make its appearance. Not only is it possible to remove the anlage from which the pars anterior, pars intermedia and pars tuberalis of the hypophysis are developed, but these structures and the pars nervosa as well can be transplanted, thus giving all possible combinations of the glands. Allen (1920) showed that the pars anterior is responsible for the control of metamorphosis through the thyroid gland. Thyroidectomy was performed upon the tadpoles of *R. pipiens*, and later a large piece of anterior hypophysis material of an adult frog of the same species was transplanted under the skin. Although the volume of this transplant was many times larger than that of the tadpole's own hypophysis, mere quantity of secretion was unable to cause any more development than occurred in those animals into which no hypophyseal tissue was transplanted. This finding was checked by removing the transplants after several months and subjecting them to microscopic study. In nearly all cases the tissues were healthy and showed every evidence of continued activity. In the few cases where the tadpoles continued in their metamorphosis microscopic examination invariably showed remnants of thyroid tissue to be present.

Allen (1927) studied a series of transplants of pars anterior, pars intermedia, and pars nervosa into *R. aurora* larvae and found that only the pars anterior exercises any influence upon metamorphosis. It was thus definitely shown that the pars anterior of the hypophysis controls metamorphosis solely through its effects upon the thyroid gland, and that it never induces metamorphosis in completely thyroidectomized tadpoles.

Many investigations besides those already discussed have dealt with the thyrotropic effect of the anterior hypophysis.

Uhlenhuth (1927) gives us a picture of the discharge of colloid during the normal process of metamorphosis. He favours the view that the colloid escapes in sparse intercellular droplets that occasionally take the stain characteristic of the colloid within the follicles. The stimulus given to the thyroid gland by the pars anterior of the hypophysis makes possible the experimental study of the cytology of thyroid function. Grant (1930, 1931 *a, b*) stimulated the thyroid glands of larval salamanders by making intraperitoneal transplants daily of the pars anterior of the hypophysis of several species of frogs, striking effects being visible after four implants, while seven daily implants caused the complete disappearance of the colloid. A study of the follicular cells during this process of release showed droplets of staining colloid within them together with clear vacuoles. Adams drew the conclusion that the colloid passes into and out of the cells in a changed, probably more diffusible, condition, being recombined temporarily into characteristically staining colloid while within the cells.

Similar observations were made by Figge & Uhlenhuth (1933) upon *Amblystoma tigrinum*; both normal and hypophysectomized animals show that there are appearances which seem to indicate a storing of the hormone. This type of study seems to offer much promise of solving the cytological factors of colloid secretion and discharge. Krichesky (1934 *a*) used the pars anterior extract, phyone, with the striking

result that ten daily injections caused complete discharge of all follicular colloid, great interfollicular vascularity and an increase of 626 per cent in the size of the glands.

Ingram (1928 *a*, 1929) followed the procedure of transplanting the pars anterior of the hypophysis of larvae of the rapidly growing species *Rana pipiens* into the body cavity of the slowly growing species *R. catesbiana* and *R. clamitans*. The work was of a qualitative type in that the transplants were inserted in considerable numbers into the coelom without taking into account the number that became established, or the exact stage of development of the donors. He showed that there is a decided increase induced in the size of the thyroid gland and in the height of the follicular cells, together with considerable discharge of the colloid contents, typical indications of thyroid activity.

The writer undertook to determine whether the hypophysis leads the way in the process of metamorphosis (Allen, 1932 *b*). This gland was taken from tadpoles of different stages and transplanted into tadpoles of a standard premetamorphic stage. It was found that the pars anterior from metamorphosing tadpoles from those in which metamorphosis had been completed and pieces of gland from adults would induce metamorphosis in the recipients, but that the pars anterior of the hypophysis of younger donors before the onset of metamorphosis had no capacity for inducing metamorphosis within the period of the experiment, even when two or more were transplanted. The conclusion was drawn that the pars anterior of the hypophysis plays the leading part in metamorphosis, that the initiation of metamorphosis depends upon its attainment of the proper stage of maturity while the thyroid gland follows its lead.

Etkin (1935 *b*) followed out this line of investigation to the degree of including primordia through to much more advanced stages. He states that there is a decrease in the thyrotropic function of such transplanted glands taken from tadpoles beyond the stages of more active metamorphosis as evidenced by a delayed metamorphic response of the recipient. He found that an excess number of transplants produces a "precocious and condensed metamorphosis". Since the paper quoted is merely a short abstract we are not in a position to discuss it.

Magdalena (1934) showed that removal of one thyroid gland and one-half the other in the case of *Bufo arenarum* caused this one-fourth glandular mass to show unusual activity at the end of 30 days. In cases, however, where he had removed the pars anterior of the hypophysis there was no histological evidence of hyperactivity.

Contrary to our own findings in *Rana pipiens* (Allen, 1929) and to the work of a number of other subsequent investigators, Spaul (1930) claimed that anterior hypophysis injections induce metamorphosis of the Mexican axolotl even in those from which the thyroid gland has been removed. On the other hand, Figge & Uhlenhuth (1933) repeated this experiment, transplanting the anterior lobe of the hypophysis to the Colorado axolotl, *Ambystoma tigrinum*, and found that there was no metamorphosis induced in those animals from which the thyroid gland had been successfully removed.

We are convinced that the hypophysis induces metamorphosis only through its control of the thyroid gland.

SUMMARY

1. The removal of the thyroid gland prevents metamorphosis in amphibians.
2. Elemental iodine administered in sufficient amount to normal, thyroidectomized, or hypophysectomized tadpoles and to those from which both of these glands have been removed brings about metamorphosis.
3. Iodine has greater potency when combined in di-iodotyrosine than in its elemental form or in inorganic salts. In thyroxin it is much more potent still and there is some evidence to show that it is still more potent in thyroglobulin of which thyroxin appears to be the essential constituent.
4. It seems quite clear that none of the metamorphosing amphibians that have been studied experimentally complete metamorphosis without the influence of iodine and that the thyroid gland, under the control of the hypophysis, is responsible for enabling the tadpole to utilize the minute quantities of iodine normally present in the environment.
5. While the thyroid hormone with its essential iodine constituent is responsible for the control of metabolism and also for metamorphosis, other substances that stimulate metabolism have no capacity for inducing amphibian metamorphosis. This has been demonstrated by experiments with dinitrophenol. On the other hand acetylated thyroxin has lost its influence on metabolism but has retained its full capacity to stimulate amphibian metamorphosis.
6. Different tissues show various degrees of capacity to absorb and retain iodine. Muscle tissue has an especially high capacity for retaining iodine. In the blood, iodine is held in the plasma rather than in the corpuscles.
7. Amphibian larvae show no response to iodine or thyroid stimulation during their early stages of development, acquiring an ability to respond only after they have reached a specific fairly advanced stage of development, differing fairly widely in different species. While axolotls show relatively low responsiveness to the thyroid stimulus, *Necturus* has not been definitely shown to respond at all.
8. There is an orderly succession of steps in metamorphosis due to differences in the threshold of response in different tissues.
9. In cases of regeneration, the younger tissues respond less actively to thyroid stimulus than do the older tissues.
10. Certain features such as the perforation of the skin by the forelimbs are not under the direct control of the thyroid hormone, while other features such as the resorption of the tail do appear to respond directly to it.
11. The anterior lobe of the hypophysis plays an essential role in the activity of the thyroid gland, which will not function in its absence. The anterior lobe of the hypophysis is not able to induce metamorphosis in the absence of the thyroid gland, but induces metamorphosis only through its influence upon the thyroid gland.
12. The anterior lobe of the hypophysis not only plays an essential role in enabling the thyroid gland to manufacture its hormone but an equally important

part in causing the thyroid gland to discharge the accumulated hormone into the blood. This has been shown by both transplantation and by the use of extracts produced in various ways. There is good evidence that the thyroid activity that leads to metamorphosis begins only when the hypophysis becomes sufficiently mature to stimulate it to activity.

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GROWTH, TIME, AND FORM

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I. THE GENERAL IMPORT OF GROWTH DATA¹

WHEN reduced to absolute dryness, a fair sample of the literature on organic growth yields a residue in which a few non-volatile platitudes are imbedded in a voluminous matrix of quantitative fact. Of all our attainments in this field, these alone are permanent. What can we do with them?

¹ This analysis of growth data is part of a programme of research supported by contribution from the Rockefeller Foundation. The extensive literature has been reviewed recently in Needham's monumental *Chemical Embryology* (1931). Some references not otherwise verifiable have been attributed to this source; others to Murray (1925, 1926), and to Donaldson (1905, 1915). I am indebted to Prof. Selig Hecht for frequent criticism and especially to Prof. Charles W. Cobb of Amherst College, for the basic mathematical operations and many helpful suggestions. However, I cannot escape responsibility for the underlying ideas and their application to specific problems.

If we weigh a growing organism at various intervals of time, any two determinations in the ascending series of weights differ by amounts somehow related to the time that has elapsed between them. When the time interval is zero, the change in weight is zero. The immediate and definite information conveyed by this experiment is that growth cannot be separated from time. But this is not all that we can learn. The increments by which the weights increase somehow are also related to the organic mass producing them. In this respect growth resembles compound interest, and the ratio increment/mass expresses the percentage productivity of the organism. Here, however, the similarity with compound interest stops. As time goes on, percentage productivity declines (Minot, 1908), and at last the organism reaches a state in which we say it is no longer growing. For many purposes this statement is sufficiently true; under certain conditions it may be entirely true. In any event, and despite the text-books of mathematics, compound interest in its accepted form is not the law of organic growth.

II. DERIVATION OF THE LAW

What then is the relation between growth and time? Without implying that we are limited to a particular case, it seems wise to begin with the chick. It is the form that has been most thoroughly explored, and its development occurs in the presence of ample supplies of food and at practically constant temperature. The data of Murray (1925 *a, b*, 1926 *a, b, c*), on White Leghorns are the most complete on a single variety, and almost without exception exhibit internal consistency. His values (1925 *a*) clearly show the relationship generalized by Minot. Fresh weight, w , increases daily by a diminishing percentage of itself. Instead of merely admitting that compound interest with its conventions fails to apply, let us determine the rate at which the interest falls. Beginning, for example, with $t = 5$ days the percentage increase during the fifth day, by inspection, is observed, empirically, to be almost precisely proportional to $1/(6^2 - 5^2)$; for the sixth day $1/(7^2 - 6^2)$, etc.; and in the general case, proportionate to $1/[(t+1)^2 - t^2]$. The fraction $1/[(t+1)^2 - t^2] = 1/2t+1$. From this the nature of $w = f(t)$ can be established.

$$\begin{aligned} \frac{dw}{dt} &= K \frac{1}{2t+1} w, \\ \frac{dw}{w} &= K \frac{1}{2t+1} dt. \end{aligned} \quad \text{.....(A)}$$

Integrated, with $\frac{K}{2} = k$, we have

$$\log w = k \cdot \log (2t+1) + C. \quad \text{.....(1)}$$

Hence if we plot $\log w$ against $\log (2t+1)$, the experimental points should fall along a straight line with slope k .

$\log w = 3.6 \log t - 0.175$, and applied the expression to a mixture of points based on Hasselbalch, and on Lamson & Edmond. Subsequently, MacDowell *et al.* (1927) compressed their own data on the pre-natal mouse as well as those of Ibsen, of Draper, and of Hensen on the guinea-pig, and of Needham and of Schmalhausen on the chick, and produced straight lines only parts of which are legitimate.

The temporal origins implicit in $\log t$ and $\log (2t+1)$ are not interchangeable, but since $2t+1 = 2(t+\frac{1}{2})$, $\log t$ must also be a linear function of $\log w$. All this, however, has not been considered important, and Murray (1925 *a*, p. 44), influenced by prevalent views, did not "attach theoretical significance to the fact that the weights can be expressed by such a simple equation".

The primary criterion by which to judge an empirical expression is not simplicity but usefulness. Unless it is mere dialect, the equation should describe all types of organic growth and enable us to extract from the data consequences hitherto submerged. These, together with the original facts, should then fuse into a system consistent within itself and with data from other fields of investigation. In this way our constants may take on the physical meanings necessary for theoretical formulation. But none of these things can happen unless we are consistent and prepared to follow rather than dictate. Perhaps we cannot continue to count time from the moment when weighing first becomes convenient; are the consequences rational or irrational? Perhaps anticipation and fact will not agree at all times and under all circumstances; can we expect such agreement unless we have first imputed to growth properties that are unknown and which it may not possess? No equation can think. $\log w = k \log (2t+1) + C$ makes no provision for discontinuity. It merely implies that as the organism advances in age experimental points separated by unit time fall closer together (Fig. 1), and that any finite value of k automatically provides for the cessation of growth at infinity. Yet even where we encounter "spurts", practical growth usually terminates well within the individual life span. Shall we condemn the equation? What if discontinuity should enable us to localize, evaluate, and perhaps understand, the mechanisms that normally bring growth to its mathematically precocious end?

How then are we to interpret the alignments in Fig. 1? Conceivably, they could be the product of chance, but if so they would hardly repeat themselves. And, if we have not discovered a most peculiar type of accident, we must assume that these weights refer to something which, during the period covered by the measurements, betrays no essential changes of category or relation. For the present such a period of stability is called a stanza of growth.

Obviously our assumption could lead to Robertson's (1923) postulate of the master reaction, only the perplexities adventitious to this idea render its application extremely difficult. Without denying that "fresh weight" directly or otherwise may reflect the peculiar properties of a controlling mechanism, we shall first consider whether our weights refer consistently and with greater immediacy to some other entity or relation of entities no less definite but quantitatively predominant. Were we dealing with the results of chemical analyses, this would be true within the limits of error. For fresh weights the suggestion does not at once commend itself. Two

organisms or parts thereof may increase in unit time by equal increments of weight, volume, or length, but not necessarily for the same reasons; again, on two occasions the same organism or part may exhibit the same incremental gain, once more for very different reasons. And yet, if the particular measurements in Fig. 1 did mean that we have been weighing essentially the same category throughout, the

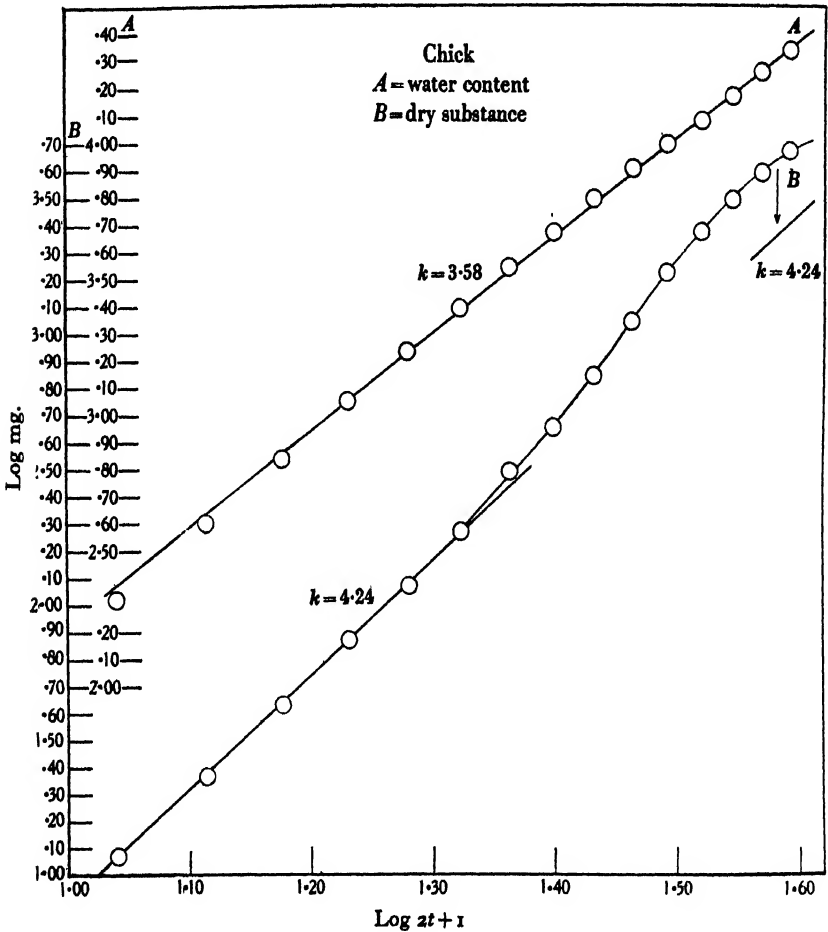


Fig. 2. Chick, based on Murray (1926 a, p. 410) and showing dominance of water over dry substance in fresh weight. The equation exposes in the category "dry substance" a distinct flexure after the tenth day of incubation.

points would fall along straight lines in no wise different from those that actually materialize.

The conditions under which a mixture of contemporaneous rates becomes superficially indistinguishable from a rate based on one chemical entity are not hard to imagine. One case would be realized by an organized mixture whose associates are related by constant proportions; a second, when the deviations of individual rates from linearity happen to compensate; a third, when an individual or group rate,

somewhat sinuous, is associated with one that is linear, but based on absolute magnitudes, greatly in excess. In this instance "contamination" by the deviating items would be completely masked by the dominant linear rate.

From this standpoint let us dissect curve *B*, Fig. 1. Murray's analyses (1926 *a*, p. 410) indicate that on the fifth day of incubation 94.7 per cent of fresh weight is water; on the nineteenth day 82.6 per cent. The dominant position of water among the substances that accumulate during embryonic growth is no novelty. What needs emphasis is that our requirement of weights reflecting the behaviour of a definite and prevalent entity or system of entities finds in water a close practical approximation. It is not surprising therefore that the accumulation of water itself should follow the linear law (*A*, Fig. 2), and that k should have a value close to k for the chick as a whole. Moreover, the influence of water on the resultant for the embryo (Fig. 1) is explicable. Not only is there quantitative superiority during the entire epoch, but dry substance itself (*B*, Fig. 2) is initially linear and after the tenth day diverges by quantities too slight to assert themselves against an overpowering domination.

IV. CONTINUITIES AND DISCONTINUITIES: SALTS

If a stanza of growth is a period of dynamic stability during which k remains constant, obviously, $\log w = k \log (2t + 1) + C$ cannot continue to fit if growth in w is affected by new increments of income or changes of metabolism. Conceivably an increase of relative income might compensate a decrease metabolic in origin or vice versa, yet in the purer categories changes of either type should manifest themselves as additions to or subtractions from the previously established rates. If such "reorganization" achieves stability at a different level, the "recapitalized" organism or part should again follow the law. Even during a period of "reorganization" the absolute magnitudes of certain types of income or storage need not necessarily be affected. In such instances the independent items should be represented in the curvilinear "reorganization rate" by submerged straight line recessives.

An especially instructive region illustrating these possibilities is found in the second half of incubation in the chick. During this time the embryo is perfecting its new system of allantoic respiration and incidentally makes use of hitherto untapped sources of income. It would be strange if "dry substance" did not behave simultaneously as though weighted by "extra-dividends". These apparently reach a maximum and after the eighteenth day decline (*B*, Fig. 2). Can we analyse the added increments and attribute specific fractions to specific processes initiated and becoming important during this period of development? If so, some of the processes when dissected out should exhibit the postulated linear rates.

Our first incision divided water and dry substance: our second separates the conventional categories, organic substance and ash. On the ninth day the allantois touches the shell and begins the more effective removal of mineral matter. The quantity absorbed from this source per day apparently increases as the allantois grows, and if not too small should exert an appreciable effect on total embryonic

ash. Murray's determinations (1926 *a*, p. 418) begin with the tenth day but were defined and extended by means of Liebermann's values. The latter clearly expose two stanzas of accumulation and, as anticipated, break upward on the tenth day (*B*, Fig. 3). In the extrapolated series, a first stanza emerges because the

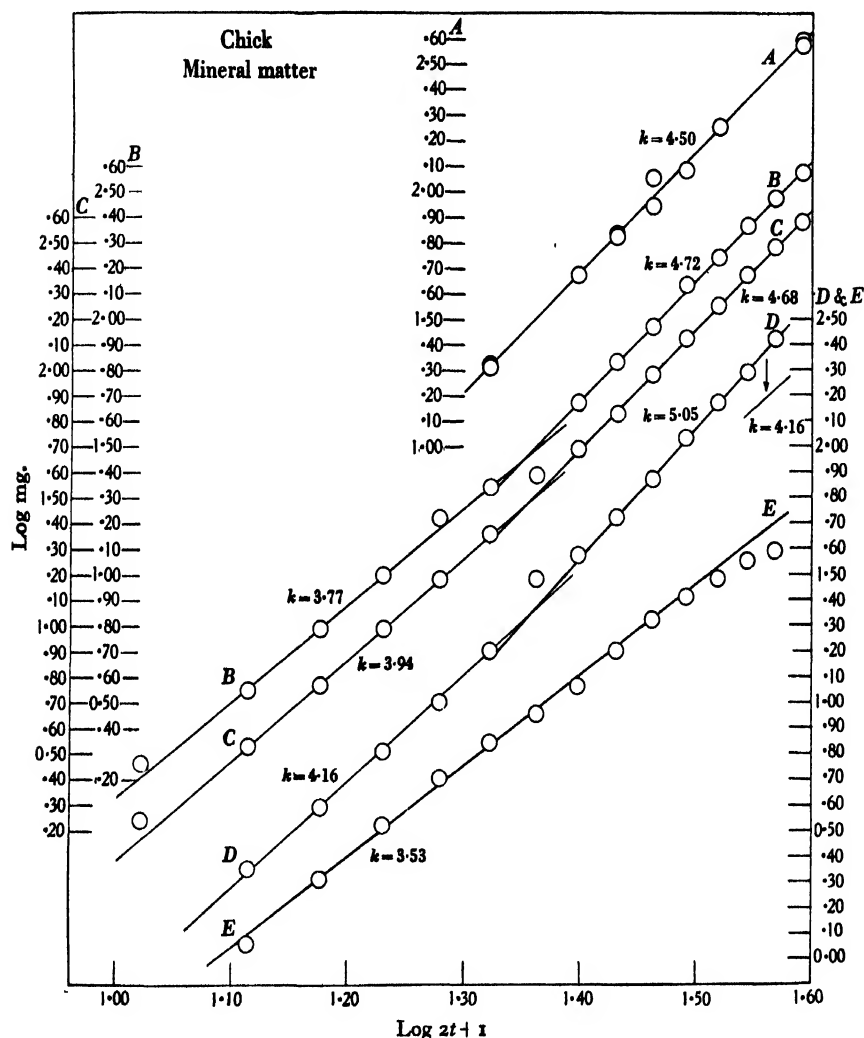


Fig. 3. Chick, mineral matter. *A* and *E*, Murray's (1926 *a*, *b*) original data on ash and chlorides; *B*, Liebermann's ash timed to conform with Murray's days; *C*, Murray's ash extrapolated to fifth day of incubation; *D*, carbonates, derived from ash by subtraction of chlorides. Murray (1926 *a*, p. 418) and Needham (1931, p. 913).

Liebermann distribution has been imposed on data calculated from Murray's Stanza II, where the two sets of observations agree (*C*, Fig. 3).

We can now subject this interpretation of the Murray-Liebermann values to a quantitative test. If accelerated accumulation of ash after the ninth day depends on an increment added from the shell, it follows that ash minus chlorides should

maintain the two-stanza form of total ash, and that chlorides alone should not exhibit an upward break on the tenth day, in both instances because the shell contains no chlorides. The first of these expectations is realized in curve *D*; the second in *E* where a change of direction, if legitimate, is actually downward. The interpretation of *E* as linear is based on the standard errors of Murray. These provide for deviations greater than those found. But we must go further.

Conceivably, the general relations shown by the curves for ash, carbonates, and chlorides could emerge if either data or interpretation or both were systematically incorrect. As suggested within the frame of Fig. 3, curve *D*, let us extend both stanzas of curve *B* to the twenty-first day which falls outside the limits of the drawing. At this time the difference in level between Stanzas I and II should correspond roughly to the total calcium carbonate moved from the shell. For several reasons this measure cannot be entirely accurate; the mineral content of the shell is only about 98 per cent calcium carbonate; some calcium is mobilized in Stanza I, and not all mobilized in either stanza reaches the embryo; some that does reach it may be transformed into phosphates or chloride; and finally the rate of embryonic calcium accession should drop as pulmonary replaces allantoic respiration. Our "curve value" for calcium carbonate absorption from the shell is certain to be too small. Actually, it amounts to 293 mg. This is about three-quarters of the amount recorded by Tangl (1908) and Tangl & Mittuch (1908) for Plymouth Rocks and compares very favourably with the shell-loss of 328 mg. calcium carbonate reported for Rhode Island Red eggs by Glaser & Piehler (1934). The average total shell-loss recorded by Murray (1925 *b*, p. 6) for the nineteenth day is 260 mg. Considering differences in material and the entirely different techniques, these accordant orders of magnitude constitute a significant comment on the validity of the equation and on our method of using it. Nor does consistency end here.

Related to one another by relatively constant proportions and dominated by sodium chloride, the chlorides conform to our definition of an organized mixture or category. Until the available supply has been incorporated into the embryo, the associated chlorides might be expected to follow the law as a unit and without complication. As depicted, $k=3.53$. Although not the only substances osmotically active, the chlorides are the most important. Hence the order of k for these salts and for water should be very similar but not identical.

V. CONTINUITIES AND DISCONTINUITIES: ORGANIC SUBSTANCE

The curve for organic substance practically parallels that for dry weight, but when we dissect out such categories as carbohydrate, fat, non-protein nitrogen, and protein, we find an array in which protein alone has the behaviour that characterizes the association as a whole. Outweighing all the other constituents, the protein complex should impose its pattern on the system. Can this pattern be analysed by means of our equation and will the analysis yield results reconcilable with other relevant but independent measurements? Let us consider the curves in Fig. 4 separately.

(1) *Carbohydrates*. The data on carbohydrate accumulation in the chick seem to call for revision. If we plot values for the entire category based on Murray's dry

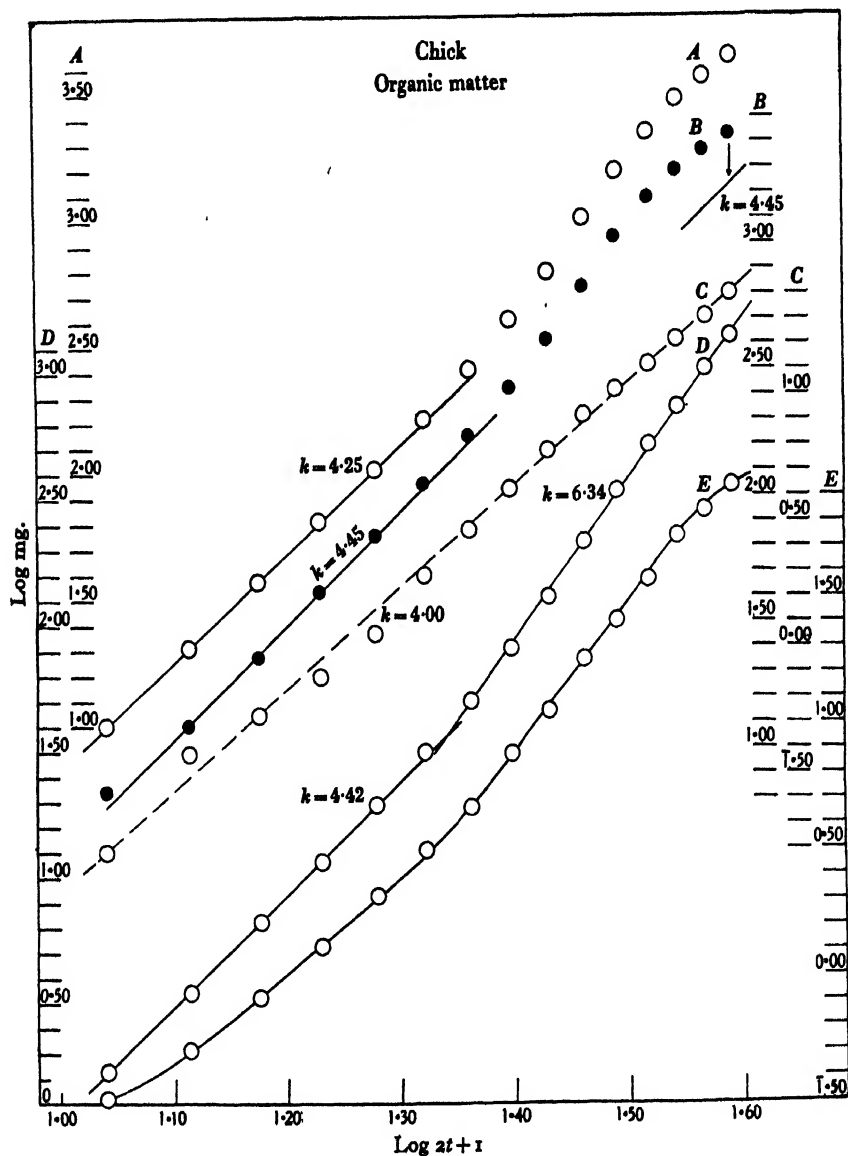


Fig. 4. Chick, organic substance. *A*, derived from dry substance, Fig. 2, and ash, *C*, Fig. 3; *B*, proteins, Murray's total nitrogen (1926*a*, Tables III and IV) minus *C*, Needham's non-protein nitrogen (1931, Table 136, p. 1075); *D*, *E*, fats and carbohydrates, from preceding data and Needham (1931, Table 108, p. 913).

substance (1926*a*, p. 410), and Needham's percentages (1931, Table 108, p. 913), the resulting curve is a fair example of the growth logistic, a form suggested in the upper terminal segments of dry weight, organic substance, and protein. When

glycogen alone is calculated from Murray's percentages (1926 *a*, p. 416), the curve may be sigmoid, or neglecting the initial point, fairly straight (C, Fig. 5). Shall we

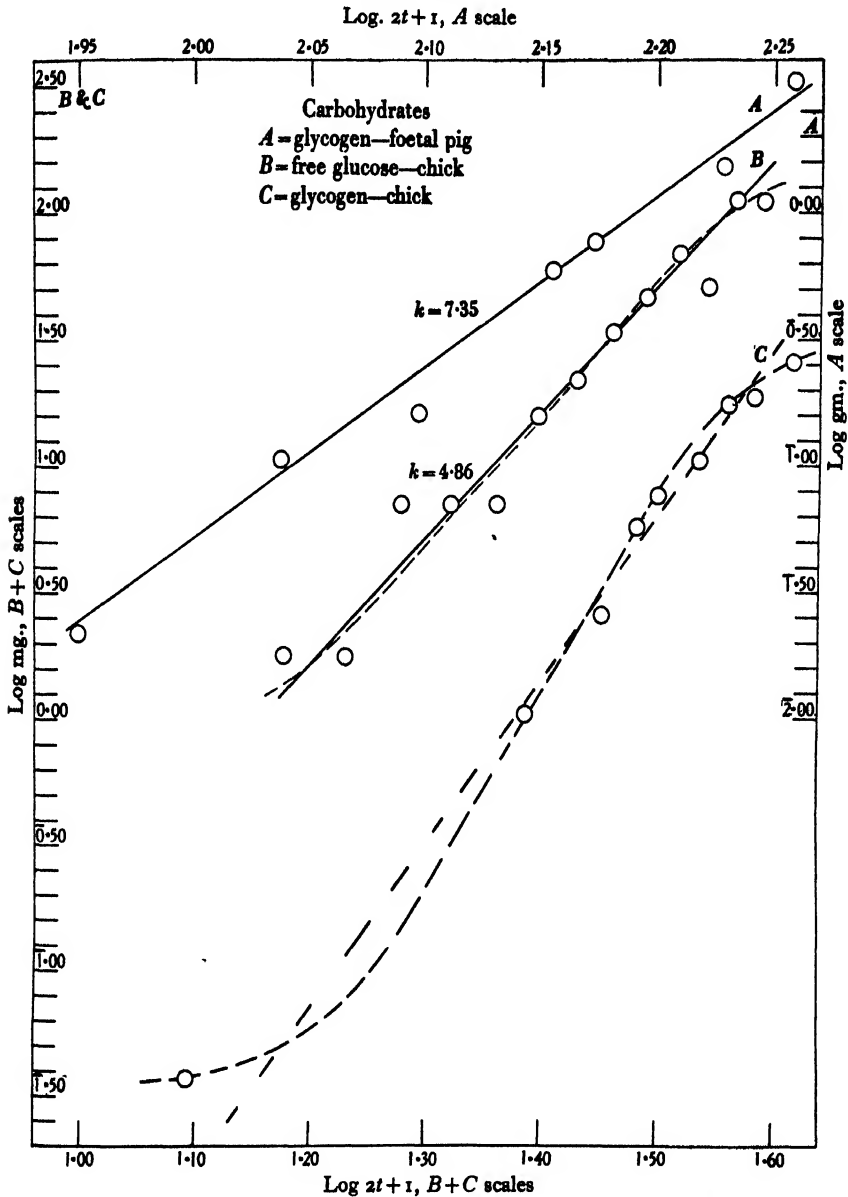


Fig. 5. Chick and pig embryo. Carbohydrates. C, glycogen, Murray (1925 *a*, Table VI), timed to conform with fresh weight; B, free glucose, recovered from curve, Needham (1931, Fig. 281, p. 1029); A, glycogen in pig embryo, Mendel & Leavenworth (1907).

infer autocatalytic synthesis? The prospect is not inviting; as evidence for autocatalysis the logistic, taken alone, is demonstrably equivocal (Kavanagh & Richards, 1934); moreover, after the fifth and sixth days of incubation, the absolute quantities

of free glucose reported by Needham (1931) are greater than our carbohydrate total, and the logistic indicated for Needham's free glucose is so nearly straight that the distinction between it and linear becomes almost academic (Fig. 5 *B*). Why then the indicated sigmoid distribution for glycogen, and for glycogen + glucose? Admittedly the case is uncertain; the two substances are represented by values powerless to insure the complete dominance of one by the other, or of both, or of glycogen alone, over the mistakes. We are dealing with integrated probabilities, and only glucose taken separately seems to approach the degree of accuracy required by the equation.

If this analysis is correct glycogen also should yield an essentially linear distribution provided we can find relatively large deposits not seriously disturbed by mobilizations into glucose and free from organized errors. This case is realized in the development of larger mammals where glycogen is a matter of grams rather than milligrams and in which the embryonic deposits are automatically protected by the transfusion of maternal blood sugar. The data of Mendel & Leavenworth (1907), on glycogen storage in the foetal pig, are shown in *A*, Fig. 5. In this instance a sigmoid curve would be highly imaginative.

(2) *Fats*. Fat metabolism during development (Needham, 1931) is as complicated as in the adult. The weights on successive days are the outcome of absorption from the yolk and of synthesis, hydrogenation and the reverse, less the amounts burnt or transformed into other categories. The details are not easy to unravel, but fortunately the molecular species involved are quantitatively interrelated, small in number, and their differences, in so far as they affect total weights, not large. As shown in *D*, Fig. 4, the points are linear, but expose an added increment after the tenth day. In this respect fat resembles ash or ash minus chlorides, and suggests that the onset of allantoic respiration is associated with events that reverberate throughout the entire embryo or complex of metabolic transactions. The series by Romanoff (1932) is shorter and less regular but will bear the same interpretation.

(3) *The protein complex*. Total nitrogen is not a satisfactory indicator for protein. We must remove non-protein nitrogen. This separation, in addition to bringing about the desired rectification of protein values, yields some insight into "residue"—a "category" profoundly affected by the chances and order of analytical procedure, and hence, for the present, biologically irrelevant. In its entirety this agglomerate furnishes a better example of the two-cycle autocatalytic curve than many alignments that have received this interpretation (cp. Needham, 1931, Table 108, p. 913 and Robertson, 1923, pp. 60 and 180). It is all the more interesting therefore that non-protein nitrogen, representing the more closely related items in the "residual" assemblage and itself perhaps dominated by lecithin, should follow the law with remarkable fidelity (*C*, Fig. 4) (data from Needham, 1931, p. 1075).

In its entirety, the protein complex behaves as if weighted by additional increments after the tenth day. Why then does it not duplicate the behaviour of the fats and carbonates? The terminal flexure suggests a certain variety and independence among the items that make up the added increments. This might be expected. The complex at all times is composed of molecular species far more diverse than those

in categories like ash, carbohydrate, or fat. With progressive differentiation the tissues presumably become more specific and efficient in the elaboration of particular substances which are tissue-true and of the greatest complexity and molecular weight. Somewhere in the egg system as a whole there must be corresponding arrears capable of accounting for the upward shift of protein weight. Should these extra-embryonic deficits have the proper quantitative attributes and together with protein destruction follow the law, the accumulation of the complex itself would be hedged in between an income and a system of expenditures, both increasing proportionately to each other. If protein storage, nevertheless, is aberrant, we must attribute this to processes which, after the tenth day and within definite limits, increase heterogeneity and molecular weight among the items that make up the total protein deposit.

Whether linear or not, extra-embryonic losses of protein nitrogen should expose an upward trend after the tenth day. Needham (1931, Table III, p. 921) lists the daily stores of true protein nitrogen outside the embryo. Determining the cumulative extra-embryonic losses we get the series of values plotted in *A*, Fig. 6. During the same 10 days, though not before, protein destruction as measured by uric acid accumulation (Needham, 1931, p. 1091) proceeds at a stabilized rate with $k=3.96$ (*B*, Fig. 6).

We now extend Stanza I of the arrears outside the embryo to the nineteenth day. The difference between this level and the actual deficit indicates excess arrears of 395.6 mg. nitrogen = 2473 mg. protein. A similar treatment of protein stored in the embryo is less reliable because the initial point of Stanza I is poorly aligned. As suggested (*B*, Fig. 4) the nineteen-day difference between Stanza I and the actual intra-embryonic level could not well be less than 997 mg. protein. Thus, the protein losses outside the embryo behave as predicted, but are much larger than necessary to account for the intra-embryonic excess stores. How can we explain the additional 1476 mg. by which the original egg capital appears to be short?

The protein incorporated in amnion, allantois, and yolk-sac wall should also be credited to the embryo. The amount can be determined indirectly from the difference between $6.25 \times$ the original endowment of 1001.2 mg. protein nitrogen in the egg, less the sum of total protein stored, destroyed, and unused, by the embryo. Total protein stored = 2793 mg. With uric acid as the indicator, protein destruction comes to 153 mg. According to Needham's (1931), Table III the unabsorbed protein on the nineteenth day = 2110 mg. Hence, at this time membrane proteins weigh 1202 mg. This is the order of magnitude required to account for the missing 1476 mg. If we add the 997 mg. of excess in embryonic storage to the 1202 mg. attributed to the membranes we charge to embryonic profit 2199 out of the 2473 mg. excess protein arrears outside the embryo. This discrepancy of 274 mg. is an allowance not too liberal for possible transformations into non-amino compounds and for experimental error. Stricter accountancy could be achieved only when we get all our information from one standardized type of egg. The chief interest of these attempts lies in the proof that within fairly narrow limits deviations from the linear law are quantitatively explicable if we follow the consequences of the equation and

discover the processes that interfere with a simple expression of the postulated proportionalities.

Our analysis therefore makes it possible to account for the upward swing of dry substance after the tenth day (*B*, Fig. 2). The minor items involved are the acceler-

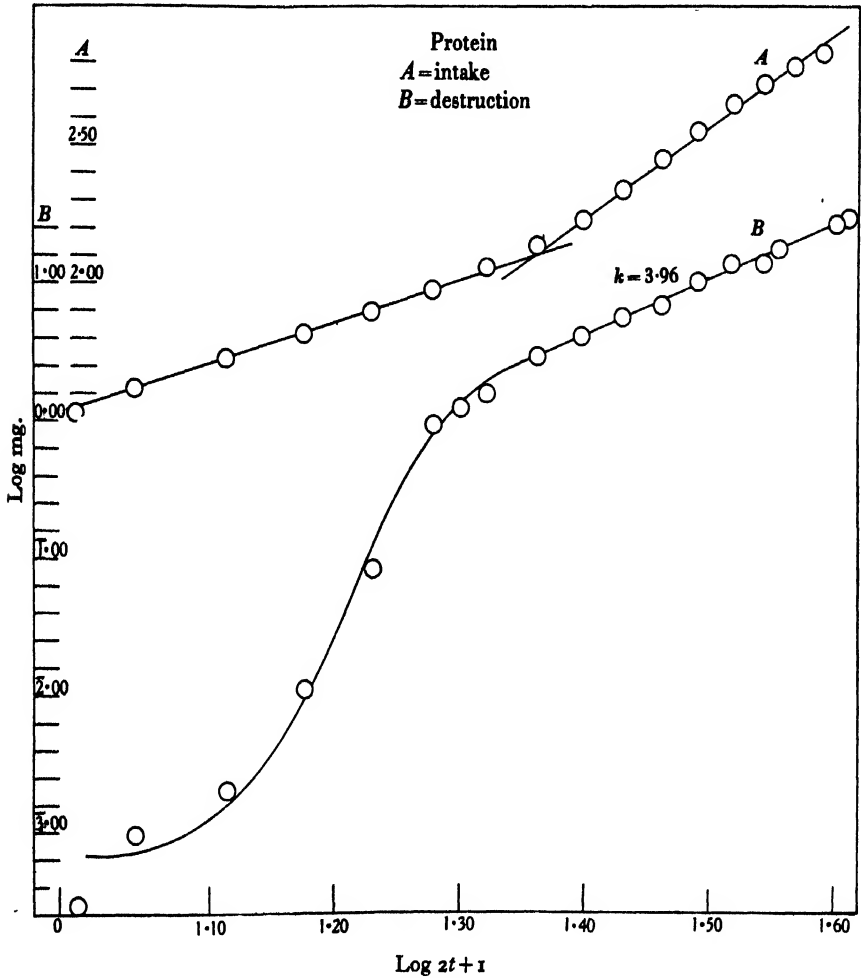


Fig. 6. Chick. *A*, protein nitrogen lost by egg through transfer to embryo with acceleration after tenth day. Based on Needham (1931, Table III, p. 921). *B*, rate of uric acid accumulation with stanza after tenth day, based on Needham (1931, Table 141, p. 1091). Relations suggest that upward swing of protein weight, stored in embryo after tenth day, is affected by molecular transformations and diversification.

ated depositions of carbonates and fats; the major item, acceleration in the storage rate of total protein. Both proteins and fats undergo a certain amount of destruction and molecular transformation. It should not surprise us that a generalized statement of these additional transactions approaches the sigmoid form, for, if it were linear, this would mean either that there is no independence among the items involved or that all are dominated by a single process. Such dominance is

unlikely since the key-note in the development of the protein complex as a whole is diversification.

(4) *Specific amino-acids and purine.* This does not mean that all individual constituents must behave like the complex in its entirety. If we can extract specific

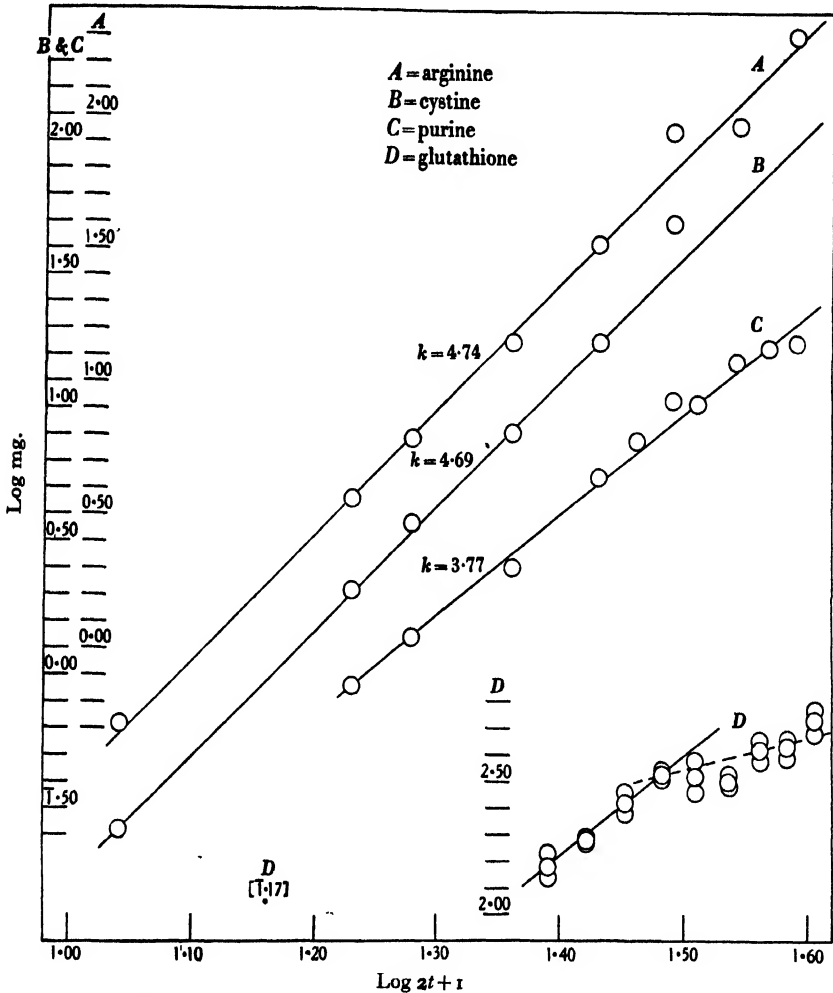


Fig. 7. Chick. Specific amino-acids and purine nitrogen. A, B, arginine and cystine nitrogen, both after Cahn (Needham, 1931). C, purine nitrogen, based on Le Breton-Schaeffer percentages (Murray, 1926 c) and true protein nitrogen, curve B, Fig. 4; D, reduced glutathione with standard errors, Murray (1926 c).

linear rates from dry substance, this should be possible also from total protein. From the Le Breton-Schaeffer percentages given by Murray (1926 c, p. 622), we can calculate the growth of purine nitrogen. The curve has the significant slope of $k = 3.77$ (C, Fig. 7). Fig. 7 also illustrates the behaviour of arginine and cystine quoted by Needham from Cahn (1931). Cystine, independently recognized by its

sulphur, behaves as if the upper terminal values were affected either by errors or by influences which at this level help to modify the entire protein category; arginine like purine nitrogen shows very fair linearity. Both have interesting implications and referring to regular constituents of chromatin may be considered index substances that expose the growth rates of genic materials. It is noteworthy that the constants for cystine and arginine, if correct, are of the same order of magnitude, and that k for purine, is identical with k for the chick as a whole. Even though we cannot commit ourselves to its entire background, Loeb's (1907, p. 80) prophetic view still appears pertinent: "nuclein synthesis is the thread by which we can find a rational way through the maze of the otherwise bewildering regulatory mechanisms characteristic of living matter: on one hand, the phenomena of growth, on the other, those of self-preservation."

VI. THE GROWTH OF EMBRYONIC ORGANS

Schmalhausen (1926, 1927) has made available data on the growth of embryonic organs in the chick. During their earliest stages, these are extremely difficult to handle and for the first four days of incubation, Schmalhausen resorted to an entirely different technique. The weights rest on indirect volumetric determinations based on sections corrected for shrinkage by linear measurements of convenient organs like the eye or lens. There were corrections for specific gravity and changes in specific gravity. Finally "paper weights" proportional to volumes were translated into "actual" weights. We may reasonably withhold judgment as to the reliability of absolute values for these stages; nevertheless the fact remains that Schmalhausen's complete series for the chick and the separated organs call for only two constants each in the linear law. Although not inherently unlikely and in no sense subversive, two stanzas for the chick as a whole are not certain. Byerly's weights (1930) for the third and fourth days make it appear as if Schmalhausen's circuitous method after all produced magnitudes closely proportional to actual weights.

Beginning with the fifth day, Schmalhausen's technique is the same as that of other investigators. Hence, it is safe to adduce his later measurements on organ growth. We are concerned with the category fresh weight, and select as examples the brain, the liver, and the heart.

Considering the mechanical difficulties even after the fifth day, the general behaviour of these data is remarkable. The low value of k for the brain may be accounted for by the exclusion of the retina and other products of the encephalon; in addition it should be noted that the constant for the heart is of an order consonant with that for water and very close to k for the entire chick. These two determinations as well as their factual bases are entirely independent. Not until this relationship had emerged was it realized that prior to the development of compensatory mechanisms, the growth of the heart—although proceeding at a totally different level of absolute weight—must necessarily keep close pace with growth in the body as a whole.

VII. GROWTH AND TIME

When organic mass is treated as a function of time it is obligatory to maintain on both sides of the equation appropriate and comparable standards of accuracy. It is no more difficult to measure an interval of time than an interval of weight, but apparently it is more difficult to know what significant time it is when we determine

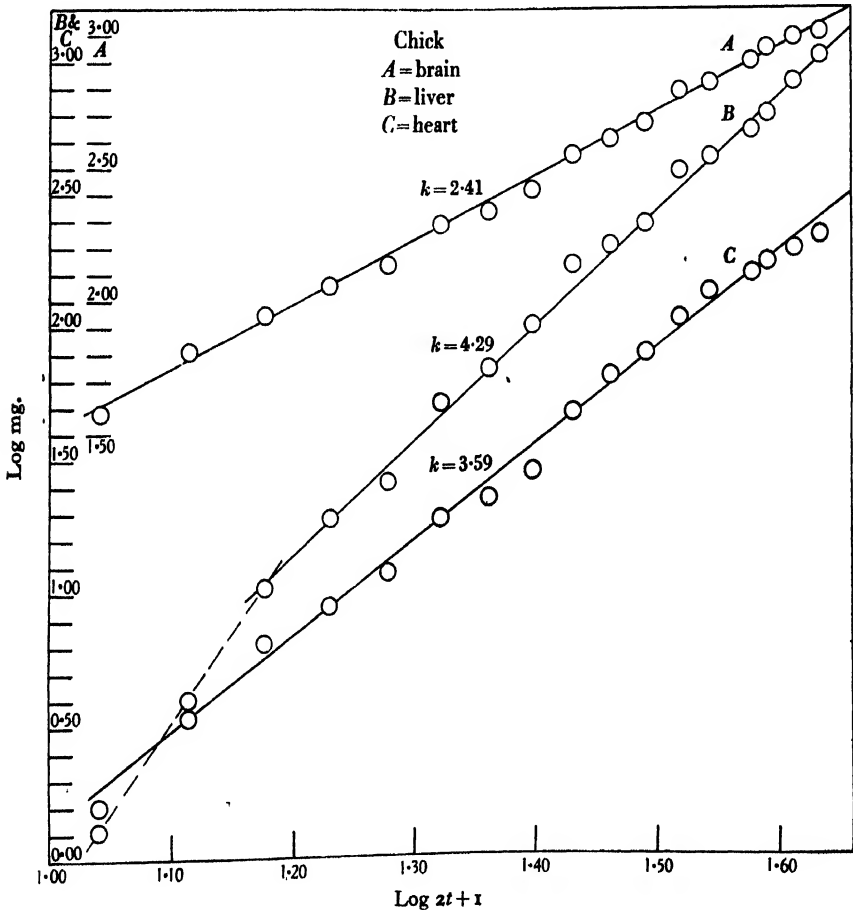


Fig. 8. Chick. Brain, liver, and heart. Weights after Schmalhausen (1927, Tables 12, 15 and 17, pp. 487, 489, and 491). Liver weights suggest two stanzas of growth.

the first of a series of weights. All the data on the chick embryo are timed from the beginning of incubation. This origin is admittedly arbitrary, but the arbitrariness is inherent in the material. The investigator, of course, can affect the interval between "oviducal" growth and the resumption of external development; yet for this intermission the expression $\frac{dw}{dt} = K \frac{1}{zt + 1} w$ reduces to zero because $dw = 0$.

Accordingly, an organism not growing in any spatial dimension is also not growing

in time and so within the limits of viability the time "lost" between laying and the onset of incubation becomes irrelevant.

But an interval of time during which an organism is not growing is totally different from an interval of growth which we choose to neglect. In mammals, especially in those with short periods of gestation, "embryo age" and "implantation age" omit different and very significant fractions of time. Consistent results can hardly be expected if we continue to impose our personal arbitrariness and date according to convenience, from conception; from implantation; from the differentiation of the embryo; and even from birth. Uniformity is essential. For studies on mammals the most defensible, and usually also convenient, "zero-hour" is conception; for the chick, it is "the onset of incubation". Ultimately comparisons with mammals may require a lengthening of the chick time-scale to allow for oviducal development; however, considering total time, our present error of 20.5 or 22 hours (Lillie, 1927) seems small, and, as we have seen, does not distort the self-consistent growth system. Moreover, the common temporal origin at the beginning of incubation, for all categories, organs, and the entire chick, produces no recognizable inconsistencies.

"Incubation-time" and "conception-time" have both been criticized. We cannot determine the precise instant when development is initiated in either case; in addition, structures like the brain, liver and heart are not differentiated or isolable even in the earliest measurable stages of the embryo. According to Huxley (1932, p. 139), growth in these organs does not begin with growth in the entirety. Hence, unless we assign each part its individual origin on the time-scale, temporal differences between whole and part or between part and part involve errors of timing that must distort our curves and the relations between them. From these difficulties, whether real or not, heterogony can hardly prove a haven of refuge.

Since no distortions are evident, the disabilities attributed to a common temporal origin may be nothing more than the after-images of misconception. In all organisms various morphological differentiations appear at different times; our weighings, at stated intervals, always include what is left of original capital endowment incorporated by the embryo or part, plus the net profits on all pertinent developmental transactions. If it is incorrect to refer our weights to the temporal region where the processes that produce a chick and those that eventuate in the parts of that chick have their origin, where else can we begin without imposing our own personal eccentricities on the material? The assumption that the part and the whole have different reference regions in time neglects the precursors of one or the other. With this elimination we step at once from the realm of genes to the realm of magic. Small wonder that the literature presents a pageant in which continuities and discontinuities frequently impersonate each other.

Neglect of the time required for cleavage implicates the same type of miracle. The familiar assertion that the dividing egg does not grow cannot be taken literally, for, granting that the underlying volumetric estimates may be true measures of mass, the total absence of growth in a developing egg calls for a system of chemical transformations that results in the increase of nothing. We have, it is true, no

satisfactory accounting for this period of development and as yet can assign no values to the growth constants of precursors. We know that chiefly water and oxygen enter the system and that carbon dioxide and water leave it. The sum of these and all other transactions could result in gain, constancy, or in loss of total weight. Whatever may be true in any specific case, the actual weight is what it is because the cleavage period results in increases of at least two items—chromatin and surface constituents. These two are probably not the only materials to be exempted when we say the egg does not grow. We advocate nothing that is not implicit in modern genetics when we postulate that every substance or structure finally realized is dependent on substances, structures, and processes found in the earliest stages of development. One precursor suffices for many products and precursors for all products are present from the beginning. As Huxley insists, this beginning cannot be absolutely localized in time, but at least we know where to look and can also decide how much precision is necessary. So long as our errors of dating are not proportionately larger than those unavoidably attached to our weights, the canons of accuracy are satisfied. Both types of error decrease as we recede from the origins. We have discovered all that we can know immediately when we have found that a percentage change in mass is related consistently to a percentage change in time. This proportionality has nothing to do with our inability to specify either the original weight of the egg or the exact instant, if there is an instant, of fertilization.

VIII. POST-NATAL GROWTH AND FAULTY TIMING

Post-natal growth is frequently used as an indicator in studies on nutrition and on the effects of inhibitors or promoters, such as hormones and vitamins. How we standardize our data and the analytical levels at which we attempt interpretation are matters ultimately of theoretical import, and immediately of practical concern. In general, post-natal weighings refer to the category "fresh weight". However, many of the advantages of the embryonic period have been lost, and exact weights, especially in large animals, are by no means easy to determine. Percentual errors may be no greater, but the materials we weigh are subject to relatively larger variations. As our animals approach maturity, the expression of genetic differences becomes more complete and the approach itself is normally carried on under conditions far from constant. Precisely how changes in temperature, moisture, diet, and in the activities of the entire organism or of constituent parts, affect the processes of growth, remains uncertain. Furthermore, in forms with large daily turnovers of food and energy, our weights may or may not include items that strictly are not, or never were, parts of the animal. Until these sources of error and their resultants have been evaluated, post-natal weights cannot have the standing of physical or chemical measurements. Even if growth after birth is essentially like that before, we should not expect from post-natal masses the precision of behaviour exhibited by embryo-chemical data. Nevertheless, we may expect the exhibition of trends not irreconcilable with each other or with data whose reliability places them in a class apart.

(1) *The domestic fowl*. The mass-time relations described by the equation cannot be expected to emerge unless we give full weight to the continuity between

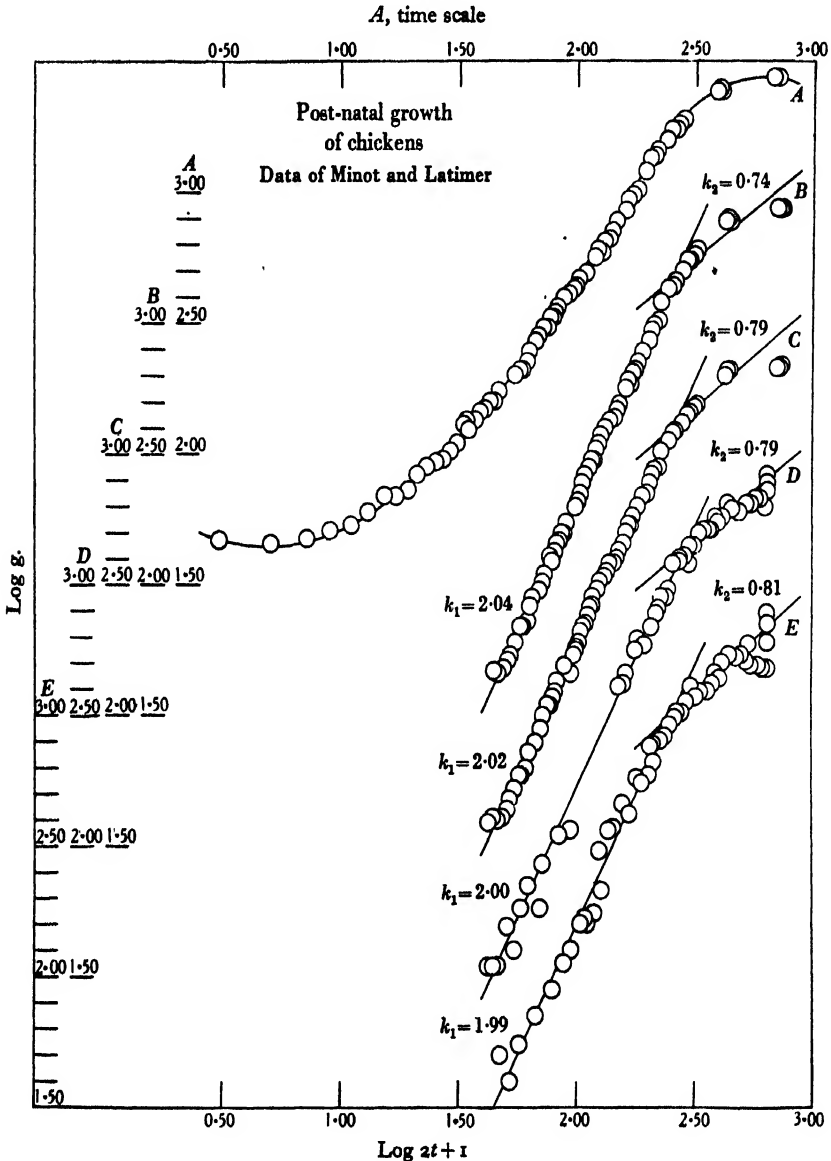


Fig. 9. Post-natal growth in chickens. A, males without pre-natal time, after Minot (1908, p. 258); B, the same with pre-natal time; C, females (Minot, *op. cit.* p. 260); D, E, White Leghorns, data recovered from Latimer (1924, Fig. 1, p. 365).

pre-natal and post-natal processes. Just as structures and substances identifiable as such only in the later stages of the embryo must be referred to precursors, themselves perhaps unknown, so the whole of embryonic time must be considered a

significant precursor of post-natal time. Indeed if growth continues to follow the pre-natal pattern, the omission of embryonic time should introduce temporal disorganization, and, unless there are compensations, the most carefully determined post-natal weights should behave like improper mass-categories and exhibit sigmoid flexures. Faulty timing is the most common error in the literature on growth. Thus, Minot (1908), in his studies on the domestic fowl, reckoned time from hatching. For females this date is called zero. The males apparently were not weighed until a day later. If we plot, for example, the values for males, without pre-natal time, we obtain the expected logistic: but if we consider the day of hatching as the twenty-first day of growth, which it is, the points fluctuate about a straight line with $k_1 = 2.04$. There is no significant change of direction until the 133rd day of total growth time.

With temporal correction, the weights read from Latimer's (1924) curves for White Leghorns duplicate the behaviour of Minot's points and supplement them in the region of the second post-natal stanza. Unfortunately the recovered data are somewhat irregular, and those of Minot too few, to establish definitively k_2 . The orders that suggest themselves in the two cases are 0.74 and 0.79 for males, and 0.79 and 0.81, for females. This localizes the onset of Stanza II in Minot's series, 1, and in Latimer's, 14 days, earlier in females than in males.

(2) *The white rat.* According to Ferry's observations (Donaldson, 1915), the white rat behaves in a comparable manner. Here again the weights straighten out when uterine time is added to post-natal. For males, k_1 remains at 2.08 practically up to the 101st day of total time. From this point on for the next 180 days $k_2 = 0.68$. Thus the first constants in these rats are essentially of the same order as in post-natal chicks and undergo similar changes in the same direction. In both types of animal the onset of Stanza II in females precedes the onset in males.

According to sample weighings recently furnished by Miss Duhring and dated 22 August 1935, the Experimental Colony Strain of the Wistar Albino Rat Colony does not yield the constants deducible from Ferry's stock. The two lines are not related and if they were it would not be surprising if prolonged and rigorous in-breeding together with improvements in diet and in the technique of handling the animals were reflected by Miss Duhring's data. In these the transition from k_1 to k_2 occurs 11 days earlier in females than in males; k_2 for males remains larger than k_2 for females; but the reverse is true for k_1 .

Differences of this type indicate the necessity for data that will enable us to differentiate between genetic and environmental influences. The importance of this distinction in sensitive material is illustrated in the experiments of Rowntree *et al.* (1934). The controls in this case descend from three pairs of Wistar-bred rats. Aside from possible exposure to other significant differences of environment, these animals were fed on a diet differing from the one provided the Experimental Colony at the Wistar Institute.

The weights recoverable from the Rowntree-Clark-Hanson control curve have

the disadvantage of being derived from both males and females in unknown and probably varying proportions. Such mixture of the sexes however cannot account for the differences between these values and those furnished by Miss Duhring. As shown in Fig. 10, $k_1 = 1.91$.

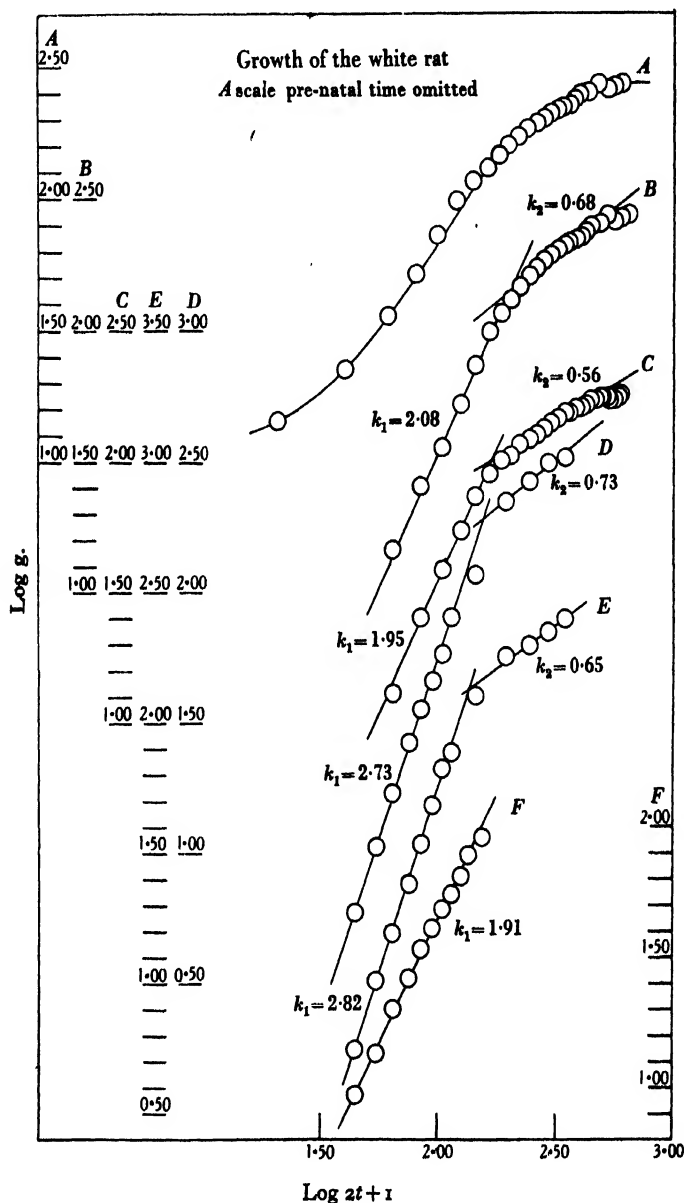


Fig. 10. Post-natal growth in the white rat. A, data of Ferry for males (Donaldson 1915, Table 65, p. 112), pre-natal time omitted; B, C, Ferry's males and females with pre-natal time; D, E, data of Miss Duhring, Experimental Colony Strain, Wistar Institute, 22 August 1935; F, data recovered from control curve of Rowntree *et al.* (*J. Amer. med. Ass.* 103, 1425-30.)

IX. THE EFFECTS OF WEANING

(1) *The Cold Spring Harbor mouse*. Divergencies definitely traceable to diet, and equally pronounced, are found in the McClendon-Street (1935), and in the MacDowell-Gates-MacDowell (1930), data on post-natal mice. McClendon and

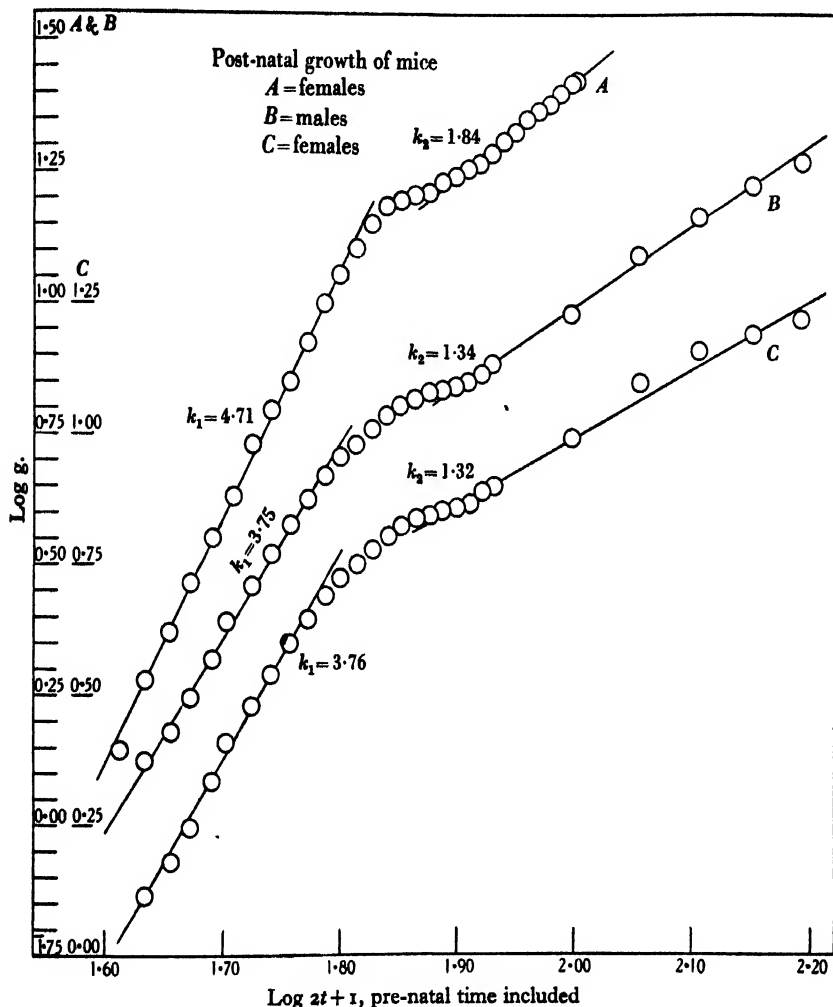


Fig. 11. Post-natal growth in mice. B, C, McClendon-Street data (1935, Table 1); A, MacDowell-Gates-MacDowell overfed female (1930, Table 1, p. 536).

Street's measurements are sexually differentiated and were made on the homozygous Bagg strain developed at the Cold Spring Harbor Station of the Carnegie Institution; the experiments of MacDowell, Gates, and MacDowell were carried on at Cold Spring Harbor, presumably on a strain closely related if not identical. Their tabulated weights are for females.

The McClendon-Street values for females, with temporal correction, yield $k_1 = 3.76$ for nine post-natal days. After this a marked shift is followed by a second alignment with $k_2 = 1.32$; for males $k_1 = 3.75$ and $k_2 = 1.34$. McClendon's explanation of the shift (private communication), is identical with the one suggested by MacDowell *et al.* (1930, p. 540). These authors attempted to remove the limiting effect of the normal milk supply. Female G.S. 2, 3 (1930, Table 1, p. 526) is the sole survivor of a litter of eight which was reduced to four immediately after birth and to one 5 days later. To prevent interference by oestrus, the mother was spayed. Under these conditions, G.S. 2, 3, became one of the six females which in these experiments achieved the highest 14-day weights. MacDowell *et al.* consider the 15th day a critical point: "New factors have suddenly become effective..." There is a "sudden change of behaviour. Up to this time the large well-fed young are markedly inactive; the eyes have opened the day before; in another 24 hours they begin to run around; they pick up solid food and begin to nibble." For these reasons the writers speak of "the break at 15 days". "This is the initiation of the natural process of weaning; during this period they take less mother's milk, but not their full requirement of solid food" (1930, pp. 540-541). As plotted in Fig. 11 the data do not bear out the imputed suddenness of the shift. The constant k_1 is 4.71 up to the tenth post-natal day and k_2 is not incontrovertibly established until the nineteenth day although the seventeenth and eighteenth might be included. Comparison with the McClendon-Street observations indicates that the experiments of MacDowell *et al.*, in addition to producing higher values for k_1 and k_2 , did actually render the transition more abrupt by postponing the onset of weaning and shortening this period in infant females by about 3 days.

It is curious, and for the present not explicable, that rats apparently reduce their weaning period to a minimum or fail to expose it altogether; it is interesting that k_1 and k_2 for the males and females of the Bagg mice are differentiated and conform relatively with the constants deduced from Miss Duhring's rat weights. In both instances, as in chicks, the change from k_1 to k_2 in females precedes that in males. This fact, with its genetic implications, together with the absence of shift in rats and the absence of both weaning and shift in chicks, makes it appear doubtful whether "weaning" with its conventional connotations can be a complete explanation for the interval of non-conformity in mice. In conjunction, mouse, rat, and chick data, seem to indicate the importance of dietary and other "external" conditions; but the sexual differences that implicate genetic factors, suggest that "weaning" may be controlled by changes in the offspring at least as much as by those in the maternal organism.

(2) *The dairy cow.* The periods of non-conformity in small animals are duplicated by similar transitions in the weights of Brody & Ragsdale (1921), on the dairy cow. These data have been analysed and interpreted theoretically, not only by their authors, but also by Robertson (1923). Since a two-cycle logistic appears to fit the observations it is necessary to examine this case with particular care.

We are not interested now in the views underlying the Brody-Ragsdale-Robertson treatments, and shall discuss only the data. The most succinct criticism

of these is given by the authors themselves (1921; especially Table 1, p. 627). The observations cover the first 29 post-natal months. Time is recorded in the uncertain "monthly" unit with zero origin at birth. "For reasons of economy", the animals were bred when 20-21 months old, so that their subsequent weights include those of the foetus and possibly may reflect also the less immediate effects of pregnancy. A correction for the foetus is possible only at term. Variability of the material is not ameliorated by the use of either large or constant numbers of animals, and the averages include items that appear rather far apart. In one instance, nine variates rated at 157 lb. include two individuals differing by 22 lb.; in another, eleven animals averaging 529 lb. include two that differ by 291 lb. For Holstein cows, mean deviation of specific items from their respective averages, fluctuates between 3.5 and 10 per cent; for Jersey cows, between 3.9 and 14 per cent. Such discrepancies, due to the nature of the material and its restricted availability, seem to disqualify these figures for theoretical uses. Indeed the final correspondence between values "calculated" on the assumptions required by Robertson and the observed values is disturbing. Thus for Jersey cows, the weights of the "last cycle" are calculated from the average weight at maturity (902 lb.) and the average weight (625 lb.) attained at 20.5 post-natal months. Since the adult weight refers to non-pregnant individuals and the earlier weight to individuals some of which could have been pregnant at the most only two weeks, the autocatalytic equation is obviously contributing something when it reproduces throughout Stanza II weights characteristic of pregnancy. Brody & Ragsdale (1921, p. 632) point out that "the observed weights before calving at 29 months of age should be greater by at least 55 lb., the weight of the calf at birth, than the calculated weights", and suggest that no conclusion "be drawn from this rather too close agreement". The difficulty receives a subtle explanation in Robertson's assumption of compensatory errors. The excess of growth "attributed by the formula to the second extra-uterine cycle" (Stanza II) is "derived from the latter part of the first extra-uterine cycle, of which the corresponding beginning portion is intra-uterine" (1923, p. 54).

Finally, the Brody-Ragsdale-Robertson analysis of monthly increments exposes only an apparent irreconcilability with the treatment of Minot and the present one. The increments rise and fall somewhat irregularly in bimodal distribution. On this rests the inference of two cycles. However, we must not overlook that within each cycle increments of growth are significant not merely because of their absolute magnitudes but chiefly by virtue of their quantitative relations to the producing mass. If we equate increment/mass against time, the Brody-Ragsdale values in each stanza exhibit the continuously falling rate implicit in our equation and characteristic of growth in general (*B*, Fig. 12).

These and most other comparable data contain no criterion that enables us to attribute the increments to specific categories. The "smooth" curve Fig. 12, has at least one major change of acceleration. Aside from the possible effects of pregnancy (*B*, between 20 and 30 months), it is probable that cumulative growth in the category "fresh-weight" is due to quite different items or to differences in the organization of items during the two stanzas that suggest themselves.

Among large animals a case with increments of growth attributable throughout to the same organic category, is realized in the antlers of deer. In these annual products we are dealing with bone and although composite, this implies a relatively

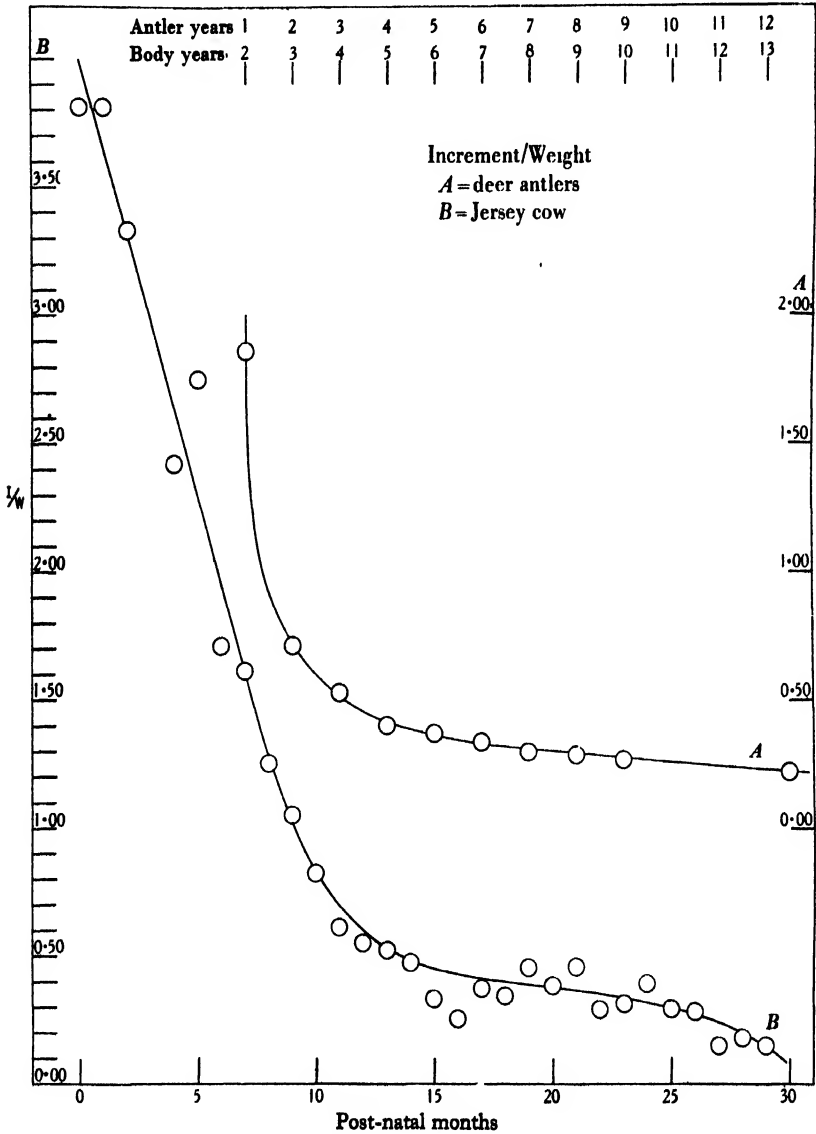


Fig. 12. $\frac{\text{Increment}}{\text{Mass}} \times \text{time}$. A, $\frac{\text{Antler weight } n\text{th year}}{\text{Body weight } (n+1)\text{st year}}$ in red deer; data recovered from curves of Huxley (1932, Fig. 29, p. 48); B, data of Body & Ragsdale on Jersey cows.

constant and definite organization of constituent organic elements and chemical entities. Unlike "cow increments" or "deer increments", antler increments may be expected to exhibit greater stability in their changing relationship to body weight.

This is illustrated in *A*, Fig. 12, based on the data of Huxley (1932) and covering a period of 12.5 years. It is a minor detail that the first pair of antlers does not

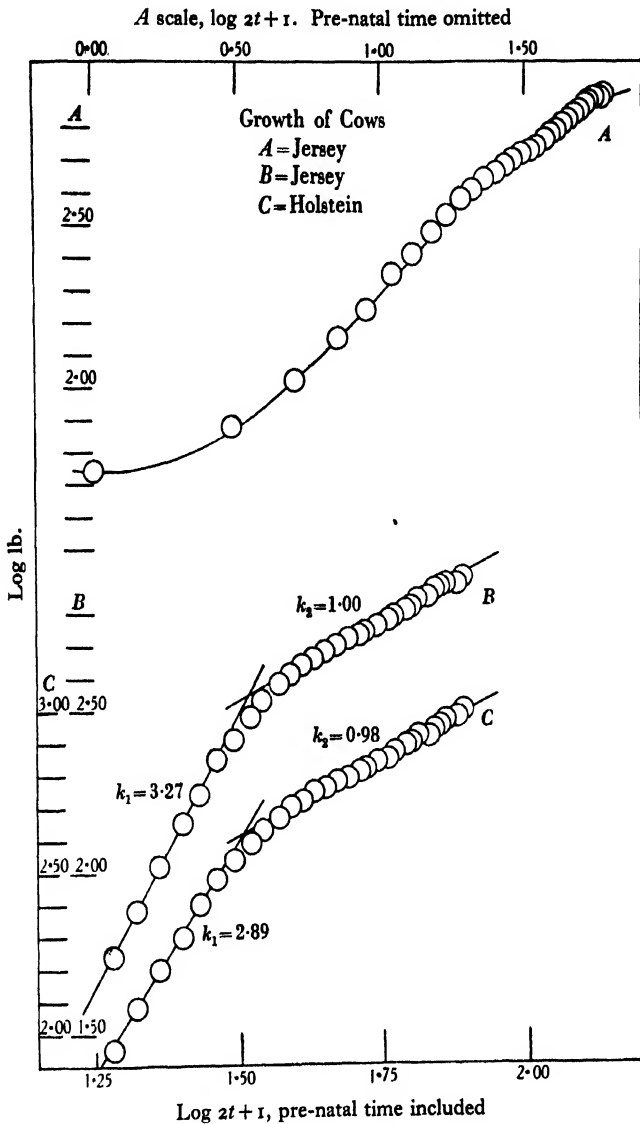


Fig. 13. $\log w = k \log (2t + 1) + C$ applied to the Brody-Ragsdale data (1921, Table 1) on Jersey and Holstein cows. *A*, Jersey cow with pre-natal time omitted; *B*, Jersey, *C*, Holstein, pre-natal time included.

appear until the second year after birth. The curve $\frac{\text{antler weight } (n)\text{th year}}{\text{body weight } (n+1)\text{st year}}$ against time is smooth and shows no significant local irregularities.

If for these various reasons we eschew theoretical analysis, we can at least inquire if the Brody-Ragsdale figures represent orders of magnitude which,

within limits inherent in the data, are calculable by means of our equation. We eliminate the adventitious temporal error by adding nine months for uterine gestation.

As shown in Fig. 13, there are no sigmoid flexures. In both types of cow, the sixth, seventh and eighth months are transitional. This period overlaps weaning and is characterized by digestive, metabolic, and perhaps other changes that might be expected to influence the rates of growth. Though incomplete and lacking in precision, this explanation appears at least as applicable here as it is to the corresponding curvilinear region of the mouse. At any rate the equation does not obscure periods of instability during which k should undergo change. It is the more interesting that for the cow as in all other cases, the points again take on a linear arrangement after readjustments are presumably complete. Moreover, since our constant for the last nine months is derived from data on pregnant cows and is a resultant of maternal and foetal constants, it is clear that our equation must deliver the weights of pregnant animals.

Table I. *Post-natal growth Jersey cow data of Brody & Ragsdale*

Age (t) (months)		Weight (lb.) obs.	Weight calc. Brody- Ragsdale	Dev. %	Weight calc.*	Dev. %
Crude	Correct					
0	9	55	55	0	55	0
1	10	76	76	0	77	1.31
2	11	105	105	0	104	0.96
3	12	140	140	0	136	2.86
4	13	174	180	3.44	175	0.57
5	14	222	222	0	221	0.46
6	15	260	265	1.92	Incal- culable "weaning" period	
7	16	302	305	0.99		
8	17	340	340	0		
9	18	376	367	2.40		
10	19	407	389	4.53		
11	20	432	Not given		413†	1.23
12	21	456			434	0.46
13	22	480			455	0.22
14	23	503			476	0.83
15	24	520	513	1.35	497	1.20
16	25	533	533	0	518	0.39
17	26	553	553	0	540	1.31
18	27	572	575	0.52	562	1.62
19	28	598	598	0	582	1.57
20	29	621	621	0	603	0.83
21	30	649	645	0.62	624	0.48
22	31	668	669	0.14	645	0.62
23	32	689	690	0.14	667	0.15
24	33	716	713	0.42	688	0.14
25	34	737	734	0.41	709	0.98
26	35	758	754	0.52	730	0.95
27	36	770	771	0.12	751	0.93
28	37	784	788	0.51	772	0.25
29	38	804	803	0.13	794	1.27
					815	1.36
						Av. 0.88

* $\log w = k \log (2t + 1) + C$, $k_1 = 3.27$.

† $k_2 = 1.00$.

For Holstein cows Brody & Ragsdale do not offer a complete series of calculated weights. Those that are given diverge more widely from the actual averages than weights calculated from $\log w = k \log (2t + 1) + C$.

The calculated and observed averages for Jersey cows together with their percentage differences are assembled in Table I. To facilitate comparison, we include also the percentual differences between the values observed and calculated by Brody & Ragsdale from the autocatalytic equation (1921, Tables III and IV, pp. 631-2).

X. A SPECIFIC POST-NATAL CATEGORY: ANTLERS

As the post-natal weights of large animals represent orders of magnitude that approximate the general pattern of growth, it is important to analyse a post-natal category structurally specific and representing a definite organic and chemical complex. So far we have considered antlers as increments of deer; despite the intermittent nature of their growth we can treat them also as categorically independent.

Antler weight can be determined with relatively high precision. However, as the data do not disclose the rates at which particular sets of antlers grow, the attempt to equate their weights with age cannot be made directly. Huxley's (1932, p. 48) observations and analyses of the red deer show that for 12.5 years annual antler weights, determined after shedding, rise smoothly to a maximum. Every pair in this ascending series requires for its full development roughly a constant fraction of a year. Thus the rate of annual antler growth increases with the age of the animal, and resembles growth resumed after starvation. In both cases the rate resumed is proportional to the producing mass although this proportionality does not remain constant. In deer the productive mass, represented by body weight, itself increases in accordance with the general equation. The constant remains at 0.79 until the animals practically stop growing between 6.5 and 7.5 years (*A*, Fig. 14).

The growth of antlers is complicated by its intermittent character and by intimate relations with the sexual system. The intermittence is easily dealt with. As the growing periods are constant, the intervals between them are constant. The time "lost" annually can be neglected because the equation applies not to antler increments (annual growth) but to total production. The measure of this in any specific year is the sum of all the antlers that have been produced up to and including that year. Accordingly, the values recovered from Huxley's curve (1932, p. 48), were "integrated". How should we expect these summations to behave?

In hinds the sexual system is not functional until the third post-natal year, whereas stags, though potentially fertile at this age, are not able to mate until weight and strength enable them to overcome the older males. As shown in curve *A*, Fig. 14, full maturity in weight is not reached until the sixth year. Conceivably, after that the quality and quantity of sex and other effective hormones undergo no further changes relative to the body as a whole, and, if so, we might expect from total antler production some reflection of the newly established stability. As indicated (*B*, Fig. 14), this rate promptly undergoes a change of direction when the animals reach maturity. At 5.9 years total age, k_1 for antler production shifts from 4.09 to $k_2 = 2.10$, and remains constant at the new level for the next five years.

XI. GROWTH AND FORM

A description limited to the relations between organic mass and time neglects one of the major aspects of growth. Hecht's (1916, p. 379) declaration that "the usefulness of an organ and the adaptedness of an organism to its environment are

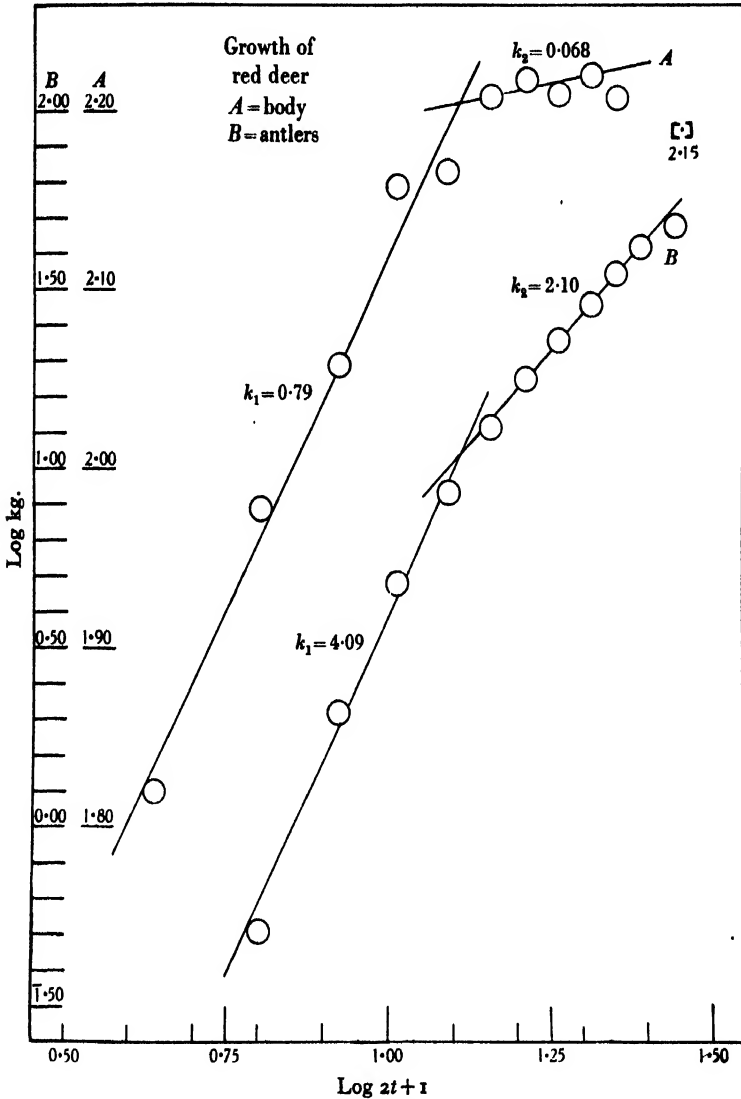


Fig. 14. $\log w = k \log (2t + 1) + C$ applied to data on the red deer and its antlers recovered from the curves of Huxley (1932, Fig. 29, p. 48).

hardly functions of their weight", may require some amendment in order to conform with certain situations; nevertheless, that "form is of prime importance", remains, in substance, undeniably true. How then is form achieved?

Initially every animal is either spherical or a modified sphere. During the earliest, often microscopic, stages, the division products of the egg become smaller with each cleavage; later they stabilize and fluctuate within the comparatively narrow limits set by the half-volume and the full volume characteristic for each type of cell. As the animal and its several organs continue to increase in volume and only reach their specific limits long after cell-size has become constant, the growing organism carries on its conquest of space primarily by increasing the number of its cells. During all this time, growth and differentiation are hard to disentangle: yet we dissociate them whenever we deal with accumulation in specific constant categories. In these, weight is a definite function of time: may we anticipate also cognate relations between time and form?

(1) *Isogonic aggregates of orthic tetrakaidecahedra*. The problem is complicated and the data needed for its analysis are just beginning to assemble. In tissues composed mainly of a single type of cell, the individual unit approximates and frequently exhibits the form of an orthic tetrakaidecahedron (Lewis, 1923, 1926, 1933). The same shape and approximations are demonstrable in the granules of a hard boiled yolk (Lewis), in certain types of starch, and in the polyhedral bodies present in the nuclei of caterpillars dead of "wilt" (Glaser, 1915; Glaser & Chapman, 1916). Under ideal conditions, with all units equal in volume and all tensions balanced, the total space occupied by an emulsoid system is incorporated without remnant into a system of rhombic tetrakaidecahedra, and is divided by minimal partitional areas (Kelvin, 1887). Quite recently, Marvin (1936) has studied aggregation in an isogonic system of orthic tetrakaidecahedra. Models made after the patterns of Matzke (1931) were stacked about a central unit. The first layer surrounding this unit contains 14 individuals; to these may be added a second layer containing 50; a third with 110; a fourth with 194, etc. If Sn represents the sum of all the units present when the layer number is n , then $Sn = 4n^3 + 6n^2 + 4n + 1$. This formula of Marvin's would be indistinguishable, experimentally, from

$$Sn = 1 + \frac{(2n+1)^3}{2},$$

since the difference between the two expressions varies only as the layer number and when $n = 20$, is 19.5 units in a total of 34,481. In its spatial aspects, therefore, aggregation in such systems of tetrakaidecahedra is represented essentially by $\log Sn = 3 \log (2n+1) - 0.3010$. This form is identical with $\log w = k \cdot \log (2t+1) + C$: however, unless the growth rings of trees or molecular films furnish the key, the outlook for a link connecting layer number with organic aggregation and time, appears too dim at this juncture to warrant more than a mere recording of the formal identity of these two equations.

(2) *Organic isogony*. Even where linear measurements are referable to specific categories, their relation to form cannot be followed through without data in other dimensions. The correlations established by Hecht (1916) for eleven species of fish indicate that the problem of form and time is soluble. In fish, final form is established early in life, and, necessarily, by differences in the growth rates of specific regions. After this, when fish of various sizes are measured in length, width, and

depth, weight equals $K \times \text{length} \times \text{width} \times \text{depth}$, and also, a constant $\times (\text{length})^3$. All that is lacking here are the ages of the fish; yet without these, the inference that form is some function of time appears secure, for if differential growth established the form, its subsequent enlargement without further differentiation is unthinkable unless all pertinent rates have become identical.

But identical rates are also found during differential growth. If we serialize the constants derived from the chick, its constituent categories and entities, there results the following array:

I			II		
	Stanza	k		Stanza	k
Fat	II	6.34	Dry matter	I	4.24
Carbonates	II	5.05	Carbonates	I	4.16
Free glucose	I, II	4.86	Non-protein N	I, II	4.00
Arginine	I, II	4.74	Uric acid	II	3.96
Cystine	I, II	4.69	Ash	I	3.86
Ash	II	4.63	Purine N	I, II	3.77
Protein	I	4.45	Fresh weight	I, II	3.76
Fat	I	4.42	Heart	I, II	3.59
Liver	I, II	4.29	Water	I, II	3.58
Organic matter	I	4.25	Chlorides	I, II	3.53

The constants in this list have very different implications but in each case refer to the sum total of the transactions involved in the accumulation of their respective categories and entities. What is immediately significant is their narrow range and the numerous instances in which a given value differs from those in its neighbourhood by less than 5 per cent. In the heart-water-chloride consonance, the agreement is much closer, and for purine and fresh weight, as well as for water and heart, we may claim identity. Groupings of two, three, or more parallel rates can be designated, isogonic diads, triads, etc. As rates may or may not change synchronously, it is obvious that the isogonic diad of one stanza may be dissolved in the next and that new diads, triads, etc., may come into being. It is likely that certain parallelisms survive until the individual stops growing and more than likely that an underlying physical-chemical system with the heart as a stirring device will prove identifiable, perhaps by means of its special consonances. Although themselves requiring further analysis, such bundles of basic isogonic rates are the mechanisms to which we may attribute the maintenance of the compatibilities during differentiation.

(3) *Heterogony*. All this is only part of the picture. No organism originates from an undifferentiated egg, and as the absolute quantities accumulated at the several rates increase, the time comes very quickly when relative acceleration or retardation have superimposed visible heterogonic results on a system at origin apparently and practically, isogonous. Thus, the foundations for all differentiations are localized in the egg and the embryologist working on the later stages is restricted to an analysis of the means of secondary morphological differentiation rather than to primary induction. In any case, materials present at the outset in small amounts, and increasing by rates, some of which are parallel, others not, or not quite, in time

must reach levels of accumulation at which the rate differences even though slight have produced noticeable or even marked effects. How far then does heterogony in the later stages depart from the practical or actual uniformities of its practically, and with respect to certain diads and triads, actually, isogonic base?

The answer is imbedded in Huxley's (1932) equation, $y = bx^k$, in which k is a constant; y represents the weight of an organ; x the weight of the animal minus y ; and b the value of y when $x = 1$. Hence when the x 's of two individuals differ by 1 per cent their y 's differ by k per cent. Obviously the stage of development is irrelevant; the same type of relationship would hold for a series of individuals fully grown and exhibiting the normal differences in adult weight. In this case, the assumption that the lighter organs and individuals in time become the heavier ones, would often, and in certain pure-lines always, be contrary to fact; Huxley's material happens to be in various stages of development; hence the assumption that the heavier individuals are also older is reasonable without being rational. A connecting law is necessary if $\Delta y/y/\Delta x/x$ is to be related with time. If this is found, it will be a step in the direction pointed out by Waddington (1933) and Needham (1932 and 1934), for both have suggested that heterogonic relationships, sooner or later, must receive definite re-orientation in the medium from which they have been so carefully abstracted.

The missing link can be forged by taking advantage of certain ambiguities in the usage of $y = bx^k$. Not only are x and y frequently linear measurements, but as a weight, x , sometimes represents the whole which by original definition is $x + y$. Moreover, the "whole" may be merely a part, in which case y is part of a part. The equation describes all these cases equally well and applies also when x and y represent the specific categories or chemical entities whose time relations we have analysed. Designating the heterogonic constant k_h , let y and x be the weights of two parts, organs, categories, or chemical entities. From A (p. 21) $dy/y/dx/x = \frac{K_y}{K_x}$, where K_y and K_x are the respective growth constants of categories x and y . Since $dy/y/dx/x = k_h$ (Huxley, 1932, p. 6) it follows that k_h is identical with the ratio between the growth constants of two categories each of which grows in accordance with our temporal equation. Necessarily k_h is constant as long as the ratio $\frac{K_y}{K_x}$ does not change; but such constant relation does not imply constancy in the absolute values of K_y and K_x . These may be entirely different in two stanzas of growth.

(4) *Magnitudes of k_h* . Let us now turn the pages of Huxley (1932) and of Needham (1934), and, omitting nothing except by chance, collect an array of heterogonic ratios. A few minutes suffice for a sample of 275, the lowest of which is 0.32 and the highest, 5.8. Without attempting to discern their specific implications in terms of categories or chemical entities, we note merely the frequencies of particular values. Within the range given, $k_h = 1$ in 11 per cent of the cases; in 14 per cent it is larger or smaller than 1 by 5 per cent or less; and of the 207 ratios that differ from unity by more than this, 110 are on the average 51 per cent larger than 1, and 97 fall short by 23 per cent. These frequencies suggest a large element of chance, especially

as the extreme values are produced by comparisons that are either curious or imply no direct connexion between the parts or measurements; thus, testicular weight, although possibly affected by both pituitary and adrenal glands, very likely is related only to a threshold weight of these structures. In these or other instances that may be kinetically dark or irrelevant, the ratio would be equally significant in reciprocal form. We cite as examples:

Reference	Material	k_h	Organism
Huxley, p. 21	$\frac{\text{Log. shoot weight}}{\text{Log. root weight}}$	= 2.65	Seedling peas
Huxley, p. 27	$\frac{\text{Log. diam. 1 ommatidium breadth}}{\text{Log. diam. carapace length}}$	= 0.32 0.40	Crustacea
Huxley, p. 27	$\frac{\text{Log. diam. nucleus (nerve cell)}}{\text{Body length}}$	= 0.4	Insect larva
Huxley, p. 257	$\frac{\text{Log. testis weight}}{\text{Log. adrenal weight}}$	= 2.3	Rabbits aged 40 days +
Huxley, p. 257	$\frac{\text{Log. testis weight}}{\text{Log. pituitary weight}}$	= 5.1 5.8	Giant rabbits, 40 days + Dwarf rabbits, 40 days +

Although it may be a matter of statistical probability whether k_h is $>$ or $<$ than 1, the fluctuation about unity rather than about some other number or about no number at all, is highly significant; it implies that over the extraordinarily wide range of data covered by Huxley and Needham, the actual growth constants, whose ratio is k_h , in each organism must be appropriate to the type and confined within limits proportionately no wider than those found in the embryonic chick. Indeed, were we to calculate, regardless of stanzaic or other relations, all possible heterogonic ratios among the known growth constants of the chick (p. 50), the resulting array of k_h values would obviously fluctuate about unity and substantially cover the range displayed by the 275 heterogonic constants gleaned from Huxley and Needham.

XII. GROWTH AND THE CONFLICT OF RATES

The several entities of the chick differ in absolute amount; all approach a common goal (p. 23); and, excepting only the most durable isogonic diads, triads, etc. do so at different rates. As a whole, therefore, the system cannot grow to infinity without developing internal crises beyond which maintenance of the unique chemical organization becomes impossible. Could we make inferences about ionic or other concentrations and velocities, the conflict in growth rates might be analysed in terms of its physical mechanisms. In principle, however, such analysis would yield little that cannot be extracted also at the chemical or organic levels to which we are restricted. Here, the conflict is manifest not in terms of a single controlling process taken alone, but directly by changing relations among the accumulated hordes in the several categories. To cite a striking and important example in the chick, the ratio water to dry substance has the following values from the fifth to the nineteenth day respectively: 17.83, 16.92, 16.09, 15.11, 14.38, 13.28, 11.98, 10.35, 8.90, 7.16, 5.84,

5.09, 4.80, 4.66, and 4.65. This 74 per cent decline represents per mg. of dry substance an almost fourfold increase in the scarcity of water.

Among students of development, Needham (1934) is the only one who seems to be fully aware that changes in the water to dry substance ratio may have significant effects. Our discussion contains much that is either explicit or implicit in his analysis (1934, pp. 87-9). The ratio is not entirely satisfactory; water is a specific chemical entity and dry substance defensible merely as the means to an end. We do not know what specific constituents, or fractions of them, are present in true solution, as hydrated colloids, or as precipitates; nor do we know how much of total water can serve as reaction medium; as a source of hydrogen and hydroxyl ions; how much is "bound", and if bound, what the properties of such water are. Until these problems are solved, the relation between water and growth can receive no precise formulation. Nevertheless, we may suspect that some of the conditions, brought on by progressively increasing drought, exercise a regulatory effect and perhaps control the practical cessation of growth.

(1) *Water and determinate growth.* In the adult (Aron, 1913) and in the less well known embryo (Glaser, 1914 a, 1916) the distribution of water is very uneven; the enamel of the teeth contains 0.2 per cent; tooth-pulp 10 per cent; the skeleton 22 per cent; kidneys, grey matter of the brain, and the vitreous humor, respectively, 83.0, 85.8 and 98.7 per cent. Omitting the secretions, certain connective and the liquid tissues, the remaining thirteen items listed by Aron (1913) contain from 69.6 to 79.3 per cent. These specific capacities for holding water are inherent in the special groupings or arrangements of dry substance. As soon as morphological differentiation enables us to isolate them, brain and cord contain the same percentages of water in the embryonic as adult frog (Glaser, 1914 a). As the ratio water to dry substance for the entire organism falls, it follows that any water appearing within the system is at once exposed to competitive absorption. The lung and kidney cells absorb more than they can keep and with access to the outer world, are responsible for systematic, and incidentally, regulatory losses.

Our basic measurements refer to absolute totals, and give no insight as to how the unequally absorbed water is "budgeted" among the various items and departments of the cell. However, it is impossible to reconcile our idea of a living cell with the belief that significant local concentrations remain at all times constant. During the process of fertilization, the *Arbacia* egg loses dissolved pigment at an accelerated rate (Glaser, 1914 c), and also decreases in volume (Glaser, 1914 b, 1924). If this implies an increase in the concentration of certain constituents, the processes in which these items take part would proceed at an accelerated rate just as processes in the chick are accelerated by heating the egg to incubation temperature. As a mechanism that increases frequency of ionic collisions, dehydration has limitations. There can be too little water and also too much; and, whether there is excess or deficiency depends on the particular process in which water is essential. However, its general decline relative to dry substance is equivalent to a decrease in certain concentrations; hence a long continued drop in the ratio of water to dry

substance must reduce certain metabolic rates from the levels necessary for growth to levels merely sufficient for maintenance.

This hypothesis can be tested, somewhat crudely. Coincident with cessation of growth and the onset of maturity, the ratio of water to dry substance should stabilize within a range characteristic for each type of organ and cell. In the rat, the ratios calculable from Donaldson's data (1915, p. 177) are given in Table IIA.

As the rat is sexually ripe 112 days after conception and senescent in three years, these data project into the first third of the period of maturity.

(2) *Water and indeterminate growth.* Turning to indeterminate growth, we should expect not only early stabilization of the water to dry substance ratio, but a ratio stabilized at considerably higher level. How much higher it should be cannot be specified. Data are exceedingly rare. As far as they go, those of Scheminzky & Gauster (1924) and of Kronfeld & Scheminzky (1925), on the trout behave as expected (Table IIB).

(3) *Water and senescence.* If stability of the water to dry substance ratio at a relatively high level is associated with continued growth and at low level with its cessation, we might expect it to deviate from the level of mere maintenance during senility. Unfortunately the literature on senescence in animals contains few measurements and these are likely to be concerned with correlations, or pathological states in man, not directly useful. If nuclear volume is accepted as an indicator of cellular water content (Glaser, 1916), the 35.8 per cent difference in the volumes of nuclei in the cervical ganglia of a child at birth and a man aged 92, quoted by Donaldson (1905, p. 329), might be significant. Another instance may be found during the development of the frog. With due reservation, the resorptive processes during metamorphosis suggest a senescent fish. Schaper's data (1902, p. 307), like the earlier ones of Davenport (1897), are not corrected for water trapped by the operculum. This error is about 3 per cent (unpublished), and may be neglected, since it is incapable of accounting for the precipitous decline of the ratio of water to dry substance during metamorphosis (Table IIC).

It would be a mistake to suppose that cessation of growth or all changes that eventuate in senescence, are unmediated results of declining water to dry substance ratios. Liquefactions, more or less localized, are not unknown. Yet, for the organism as a whole and for many of its parts, a falling ratio is the rule. The most reliable data are to be found in the literature of agriculture. Identifying "ripening" with "senescence", every annual plant as a whole, and many others with respect to specific organs, complete their life cycles in a single season. In crop plants, the economic motive has led to careful studies of the influence of both internal and external conditions on yield; our interest is in the behaviour of the water to dry substance ratio under circumstances essentially normal for each type of plant. The ratios calculable from data on maize plants (Henry & Morrison, 1917, p. 14) and oat plants (Johnson, 1890, pp. 224-5), are given in Table II D and E.

If growth is controlled not by an individual item or process but by changing interrelations among several items or processes, certain categories not immediately affected by these changes should continue to grow after other categories have ceased.

Table II. *Water to dry substance ratios*

A. White rat				
Conception age: days	Body	Muscles	Heart	Eyes
22	7.54	8.34	6.24	12.51
29	3.97	5.17	5.94	8.61
42	2.20	3.42	4.55	6.57
64	2.39	3.25	3.76	4.81
94	2.03	2.96	3.63	4.88
172	2.10	3.11	3.77	4.26
387	2.17	3.20	3.46	3.94

B. Trout		
Conception age: days	Water/dry substance	Remarks
30	5.56	Hatched
38	5.46	
45	5.00	
51	6.40	
61	6.78	
75	6.17	
99	6.10	

C. Frog tadpole		
Conception age: days	Water/dry substance	Remarks
6	2.00	External gills disappearing
11	5.64	
14	10.84	
17	14.40	
20	17.10	
23	15.93	Formation of operculum
27	18.38	
32	18.66	
40	19.06	
54	12.58	
78	11.91	Metamorphosis begins
83	9.68	
87	7.47	
183	5.30	
548	3.87	
1825	3.23	Completely grown

D. Maize		
Date	Water/dry substance	Remarks
July 24	6.16	4 ft. high
Aug. 6	7.38	1st tassels
Aug. 28	4.32	Silks drying
Sept. 10	3.34	Milk stage
Sept. 24	2.17	Glazing stage
Oct. 1	1.89	Silage stage
Oct. 8	1.36	Ready to shock

E. Oats		
Date	Water/dry substance	Remarks
June 19*	3.95	4-5 leaves
June 29		
July 8	2.79	Full bloom
July 28	1.76	Ripening
Aug. 6	0.54	Ripe

* Age 58 days.

F. Potato			
Date	Plants	Tubers	Remarks
July 3	9.06	5.20	Stems dry. Leaves dead (omitted) Leaves and stems omitted
Aug. 4	6.16	4.21	
Aug. 28	5.22	4.37	
Sept. 20	3.61	3.25	
Oct. 10	3.55	4.05	
Oct. 23	3.36	3.36	

G. <i>Collinsia bicolor</i>			
Date	Stems	Roots	Remarks
June 11	15.37	3.03	Flowers
June 20	6.87	3.03	
June 30	2.58	3.73	
July 12	4.77	3.73	
July 22	2.58	4.23	
July 29	1.08	2.74	Seeds fallen, some stems green
Aug. 25	0.42	1.50	Dead

H. Human brain	
Age	Water/dry substance
Foetus 25 cm.	10.23
1 day (post-natal)	8.34
1½ months (post-natal)	7.19
3½ months (post-natal)	6.23
7 months (post-natal)	5.11
8 months (post-natal)	5.00
11 months (post-natal)	5.22
1½ years	4.66
2½ years	4.60
3½ years	4.42
4 years	4.08
6½ years	4.00
20 years	3.44

I. Beef muscle	
Age	Water/dry substance
Foetus 10 cm.	9.41
Foetus 20 cm.	9.52
Foetus 30 cm.	9.00
Foetus 40 cm.	7.06
Foetus 50 cm.	5.25
Foetus 9 months	4.03
Adult	3.44

Thus long after a "man" has stopped growing, his hair, sex cells, skin, and adipose tissue, continue. By the same token, senescence in a specific part and in the whole may proceed at relatively independent rates. Here again the best evidence comes from plants. We calculate from data on the potato, and on *Collinsia bicolor*, quoted by Dehérain (1902, pp. 345 and 314) (Table II, F and G). The list could be considerably lengthened. On account of its peculiar interest we add only one more item. According to Liebermann (1926) metabolic turn-over in unit time per gram is practically identical in muscle and brain. If then the immediate and indirect relations here postulated are real, we should expect the water to dry substance ratio to stabilize within similar if not identical ranges. Ratios derived from the data of Aron (1913, pp. 627 and 632) on beef muscle and the human brain are presented in Table II H and I.

XIII. SUMMARY

1. The equation, $\log w = k \cdot \log (2t + 1) + C$ is a peculiar modification of the compound interest law. It is derived from growth in fresh weight of chick embryos, and applies to fresh weights of the domestic fowl and of the smaller as well as larger domestic mammals. For the dairy cow, weights are calculable from time, with an error of less than 1 per cent.

2. The circumstances under which k can remain constant are analysed. A period of constancy is called a stanza of growth. Stanzas for the whole and the part or for different parts, may or may not be synchronized. The conditions under which a particular category terminates a stanza of growth can be foreseen from the circumstances that assure the constancy of k .

3. In the category of fresh weight, water is the quantitatively dominant entity. The equation describes also other categories: (a) when dominated by specific entities; (b) when their quantitative organization is constant; (c) when applied to specific isolated chemical substances. This and the relatively independent behaviour of unit categories or entities makes it possible to calculate the amount of calcium moved from the shell by the developing embryo; and to account for the upward swing of dry substance after the ninth day of incubation. The only specific post-natal category dealt with, is "antlers" in deer. Such analysis is possible since, under certain conditions, the time "lost" in intermittent growth, becomes irrelevant.

4. The effects of temporal errors introduced by faulty timing are discussed and illustrated. According to the equation, any finite value of k automatically provides for the termination of growth at infinity. Actual cessation is mathematically precocious, and implicates heterogony.

5. By means of $y = bx^k$, the functional relation between organic mass and time can be extended to include also form, since the heterogonic constant $k_h = \frac{K_v}{K_x}$ or $\frac{K_x}{K_v}$.

6. Heterogenous growth may be considered as imposed on a system in certain respects practically or actually isogonous at origin. Parallel rates whether limited to a single stanza or surviving several are designated according to the number of rates

involved, as isogonic diads, triads, etc., and are responsible for the maintenance of the compatibilities during heterogonic growth.

7. Among the heterogonic relationships is one that cannot at all times conform to $y = bx^k$, since it involves the mixture "dry substance". Excepting the frog tadpole prior to metamorphosis, the ratio of water to dry substance falls during growth and stabilizes at maturity. Where growth is indeterminate, stabilization occurs at a level higher than for determinate growth.

8. Certain kinetic implications of the water to dry substance ratio suggest that growth, maintenance, and senility, are related to changes in the rates of the several metabolic transactions. This hypothesis is tested by an appeal to data on the frog, the white rat, man, and a variety of crop plants. In all the plants the onset of senility demonstrably associates with a fall of the water to dry substance ratio below the maintenance level of maturity.

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ÜBER PERIODISCHE FORMBILDUNG BEI PFLANZEN

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I. ONTOGENIE

(1) *Periodische Blattentfaltung aus der Knospe*

FÜR den Pflanzenkörper ist charakteristisch, dass sich gleichartige Organe, vor allem die Blätter, in grosser Zahl wiederholen. Die Blätter stehen längs einer Sprossachse in grosser Zahl hintereinander; sie erscheinen am fortwachsenden Ende der Sprossachse in der Zeit nach einander. Diese Periodizität zu erforschen ist eine besondere Aufgabe des Botanikers; diese Periodizität liefert ihm zugleich wertvolle Hilfsmittel zur Erforschung der Ontogenie des Sprosses.

Zu solchen Untersuchungen eignen sich in erster Linie Laubsprosse, welche längere Zeit gleichmässig fortwachsen. Ihre Endknospe ist umhüllt von jungen heranwachsenden Laubblättern; jedes Mal wenn ein Laubblatt sich entfaltet, wird ein jüngeres Laubblatt sichtbar. Man kann durch einfache Beobachtung des

Sichtbarwerdens, der Entfaltung oder der Verfärbung den Altersabstand der auf einander folgenden Blätter bestimmen (Sch.¹ 1916, S. 19); oder man misst im Abstand von ein bis drei Tagen die sichtbaren Blätter auf einen halben Millimeter genau und findet den Altersabstand als horizontale Verschiebung gleicher Wachstumskurven (Sch. 1914, S. 239; 1923, Fig. 1; 1927, S. 327; 1929, S. 757). Wir nennen den Altersabstand aufeinander folgender gleichartiger Organe das Plastochron (Askenasy, 1880). Das Plastochron schwankt zwischen Bruchteilen eines Tages und mehreren Monaten; für *Lathyrus latifolius* finden wir zwei bis drei Tage, für die grossblättrige *Coccoloba grandifolia* zwei Monate (Sch. 1916, S. 28). Im Altersabstand der auswachsenden Blätter spiegelt sich wieder das Plastochron des Vegetationspunktes, der periodisch neue Glieder der Sprossachse und neue Blätter anlegt.

Das verschiedene Aussehen fortwachsender Endknospen wird wesentlich bestimmt durch die Anzahl der Blätter, die gleichzeitig auf verschiedenen Stufen der Entfaltung sichtbar sind. Die grosse Seerose *Victoria cruciana* liess im Gewächshaus des botanischen Garten München im Zeitabstand von 2.1 Tagen neue Blätter auftauchen; vier bis fünf wachsende Blätter waren gleichzeitig an der Wasseroberfläche sichtbar; die Entfaltung eines einzelnen Blattes konnte während neun Tagen verfolgt werden. Dadurch dass die Blätter in ihren Wachstumszuständen dicht aufeinander folgen, entsteht der Eindruck rascher Entwicklung. Dagegen entfaltete der Baum *Artocarpus incisa* seine grossen Blätter im Abstand von zwanzig Tagen; das sichtbare Wachstum begann jeweils mit dem Durchstossen einer kegelförmigen Nebenblatthülle; neun Tage später war das Blatt entfaltet und nach einer scheinbaren Ruheperiode von elf Tagen wurde erst die Spitze des folgenden Blattes sichtbar. So entsteht der Eindruck einer langsamen Entwicklung (Sch. 1916).

Solche einfache Lebendbeobachtungen sind deutliche Hinweise auf verschiedene Typen der dauernd embryonalen Knospenzentren, der Vegetationspunkte. Beim Zergliedern der Knospen kleinblättriger Wasserpflanzen wie *Helodea* findet man vierzig bis fünfzig Blattquirle, die mit sehr kleinen Entwicklungsabständen aufeinander folgen. Der lange zapfenförmige Vegetationskegel verliert bei der Abgliederung eines Stengelgliedes und Blattquirls bloss etwa einen Zehntel seiner Masse. Er hat sich rasch wieder regeneriert, während der junge Blattquirl erst wenig in der Ausbildung voran geschritten ist. Das Teilungsverhältnis des Vegetationskegels in regenerierenden Vegetationskegel und in Stengelglied mit Blattquirl ist massgebend für den Typus der Knospe und der Knospenentfaltung. Ein entgegengesetzter Typus ist verwirklicht bei den lebenden Kieselsteinen aus der Gattung *Mesembryanthemum*. Der assimilierende Körper besteht aus zwei seitlich zur dickwandigen Röhre verbundenen Blättern; von Zeit zu Zeit werden diese von innen her verdrängt durch ein folgendes Paar und fallen als verschrumpfte Hülle ab. Gleichzeitig am Spross vorhanden ist das assimilierende Blattpaar, ein heranwachsendes Blattpaar und der blattbildende Vegetationspunkt. Der breite niedrige Vegetationskegel von *Mesembryanthemum* wird fast ganz verbraucht zur Bildung

¹ Sch. = O. Schüepp.

eines Stengelgliedes und Blattpaares; bis der kleine Rest wieder zur Blattbildung fähig wird, hat das vorangegangene Blattpaar einen grossen Entwicklungsvorsprung erreicht (Sch. 1916).

(2) Wachstumsmessung nach Askenasy

Verschiedene Autoren haben versucht, die Periodizität des Knospenwachstums zu Wachstumsmessungen an Vegetationspunkten und jüngsten Anlagen zu benutzen (Carl Nägeli, 1855; Askenasy, 1880; Westermaier, 1881; Klein, 1884). Trotz der ausgezeichneten kritischen Darstellung der Methode durch Askenasy hat dieselbe die verdiente Beachtung noch nicht gefunden. Als Beispiel betrachten wir Messungen an den Blättern der Brennessel *Urtica dioica* (Fig. 1). Es wurden

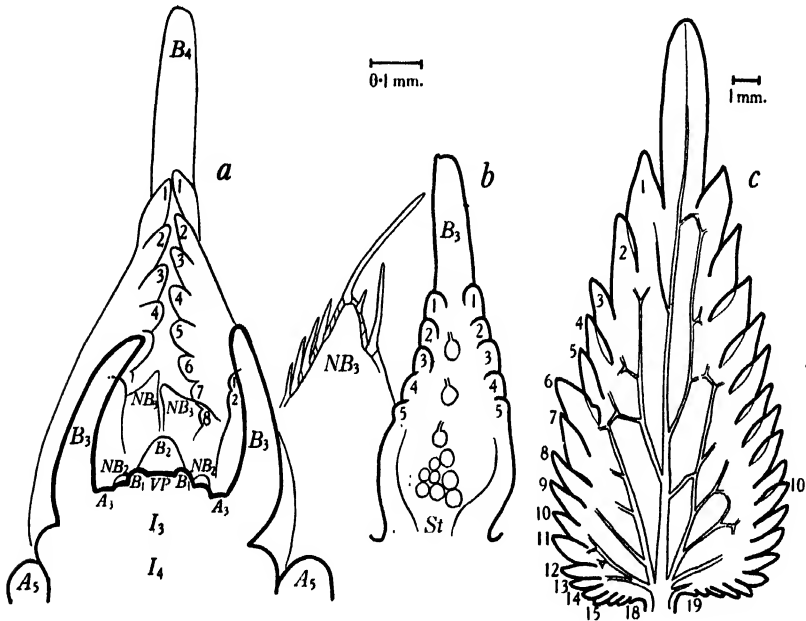


Fig. 1. *Urtica dioica*. (a) Längsschnitt der Knospe F. B, Blätter; NB, Nebenblätter; A, Achsel-sprosse; I, Internodien. (b) Blatt und Nebenblatt 3 der Knospe H. (c) Blatt 6 der Knospe A.

acht Endknospen A bis H von noch nicht blühenden Stengeln des gleichen Standortes untersucht und ausgemessen, teils durch direktes Abmessen, teils nach Zeichnungen in 25, 50 und 200facher Vergrösserung. Spross A ergab mit einigen durch die Präparation verursachten Lücken folgende Zahlen in Millimetern:

Internodien	—	0.06	0.15	0.33	0.86	—	—	16	26.5	38	49	85
Nebenblätter	0.015	0.225	0.65	1.84	6.2	—	—	9.5	10.0	10	10	10
Blätter	0.100	0.385	0.90	—	8.3	—	44	75.5	108.0	122	121	91

Der Altersabstand der gemessenen Stadien ist gleichmässig ein Plastochron. Die Internodien des Stengels befinden sich bis zum zwölften Plastochron im Wachstum; die Nebenblätter sind mit acht, die Laubblätter mit zehn Plastochron ausgewachsen.

Um das gesamte Material zusammen zu fassen ordnen wir die acht Sprossen nach dem Zustand des Vegetationspunktes und der jüngsten Blätter in eine Reihe und finden beispielsweise für die Blattlängen:

	F	B	E	G	H	D	A	C
Jungstes Blatt	0·010	0·010	0·015	0·045	—	0·050	0·100	0·075
Zweitjungstes Blatt	0·090	0·095	0·130	0·150	—	0·235	0·385	0·400
Drittjungstes Blatt	0·300	0·40	0·55	0·48	0·70	0·83	0·90	1·35
Viertjungstes Blatt	0·98	1·24	—	1·24	1·58	—	—	2·08
Fünftjungstes Blatt	2·4	—	—	—	3·88	—	8·3	—

Jeweils nach Ablauf eines Plastochrons ist der Vegetationspunkt in seinen Ausgangszustand zurückgekehrt; aber das vorher jüngste Blatt ist zum zweitjungsten geworden. Die aufeinander folgenden Zeilen unserer Tabelle lassen sich

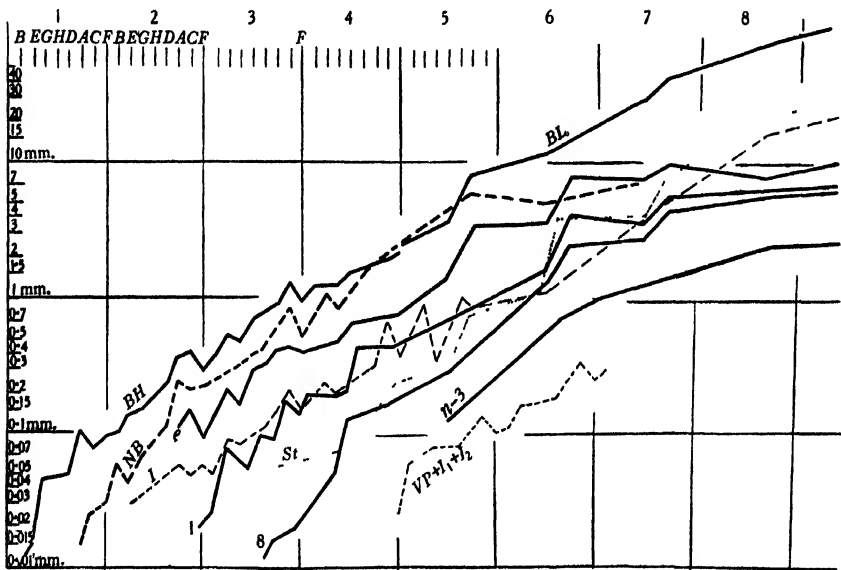


Fig. 2. *Urtica dioica*. Wachstumskurven der Blätter. Abscissen: Zeit in Plastochron 1 bis 8, eingeteilt in Achtersplastochron für die Knospen FBEGHDAC. Ordinaten: Längen dargestellt durch ihre Logarithmen. BH, senkrechte Blatthöhe; BL, Blattlänge; NB, Nebenblätter; e, Endzipfel; 1 erster, 8 achter, $n-3$ drittletzter Seitenzipfel; I, Internodium; St, Blattstiel; $VP+I_1+I_2$, Vegetationspunkt mit jüngsten Internodien.

lesen als eine fortlaufende Zahlenreihe, welche die Entwicklungsstadien eines typischen Blattes repräsentiert. Für die Zeichnung von Wachstumskurven würden wir ferner voraussetzen, dass unsere acht Sprosse F bis C Stadien darstellen, die im durchschnittlichen Altersabstand von ein Achtersplastochron aufeinander folgen (Sch. 1923, S. 45). Fig. 2 fasst eine Anzahl solcher Kurven zusammen. Zeiteinheit ist das Plastochron eingeteilt in acht gleiche Abschnitte. Als Längeneinheit sind am linken Rande Millimeter angeschrieben und die Logarithmen der Millimeterzahlen abgetragen. Das beruht auf den folgenden Tatsachen und Überlegungen (Blackman, 1919; Sch. 1914, 1920, 1923):

Betrachtet man nicht nur den letzten Teil eines Wachstumsablaufes, wie er sich sichtbar und der Lebendmessung zugänglich ausserhalb der Knospenhülle abspielt, sondern den Gesamtverlauf des Wachstums vom Vegetationspunkte ab, beispielsweise die Längenzunahme eines Blattes von 0.01 auf 120 mm., also ein Wachstum auf das Zehntausendfache der Anlagegrösse, so erweist sich die Exponentialkurve als typische Grundform der Wachstumskurve. (Die bekannte *S*-Kurve von Robertson (1913) passt nur für den letzten Wachstumsabschnitt.) In der Exponentialfunktion

$$y = y_0 \cdot e^{rt},$$

bedeuten y die Grösse zur Zeit t , y_0 die Anfangsgrösse zur Zeit $t=0$, e die Basis der natürlichen Logarithmen und r die relative Wachstumsgeschwindigkeit oder den "efficiency index". Die Exponentialfunktion folgt theoretisch aus der Annahme, dass die Wachstumsleistung in erster Linie abhängig ist von der Menge der wachsenden, an ihrer eigenen Vermehrung arbeitenden Substanz. Durch Logarithmieren geht unsere Formel über in

$$\log y = \log y_0 + rt \cdot \log e,$$

d. h. trägt man im Koordinatensystem statt der gemessenen Grössen die Logarithmen derselben auf, so wird die Exponentialfunktion durch eine Gerade dargestellt und jede Besonderheit einer Wachstumskurve zeigt sich als Abweichung von der Geraden. Wir definieren mit Askenasy (1880) die Wachstumsgeschwindigkeit als relative Wachstumsgeschwindigkeit

$$r = \frac{\log y_2 - \log y_1}{(t_2 - t_1) \log e}.$$

Sie wird in unseren Kurven dargestellt durch die Steigung eines Kurvenabschnittes, den Tangent des Neigungswinkels. Logarithmische Wachstumskurven lassen auf den ersten Blick die entwicklungsphysiologisch wichtigen Beziehungen hervortreten.

Das Pflanzenmaterial, an dem unsere Messungen durchgeführt wurden, zeigt Variabilität in Bezug auf die Stärke der Vegetationspunkte und der aus ihnen hervorgehenden Organe. Variabel ist auch das als konstant vorausgesetzte Plastochron; ungleich sind die als gleich vorausgesetzten Altersabstände der acht verschiedenen Knospen; ungenau sind alle einzelnen Messungen. Alles das äussert sich im Zickzackverlauf der Kurven. Aber alle diese Störungen sind nicht so gross, dass nicht trotzdem ein klares Gesamtbild des Wachstums entstehen würde. Denkt man sich die kleinen Unregelmässigkeiten des Kurvenverlaufes ausgeglichen, so ergibt sich ein typisches Bild der Unterschiede im Wachstum der verschiedenen Organe.

Im Einzelnen bemerken wir Folgendes: Die Blatthöhe *BH* steigt im ersten Plastochron ausserordentlich rasch; zum eigenen Wachstum kommt hier die Verschiebung des Zellmaterials durch die innere Wachstumsbewegung des Vegetationspunktes hinzu. Das Nebenblatt *NB* erscheint später als das Blatt, wächst rascher

in die Länge, erreicht die Blattlänge im fünften Plastochron, geht aber bald darauf in den Dauerzustand über. Die Gliederung des Blattrandes macht eine weitere Analyse möglich. Die ersten oberen Randzähne erscheinen am Ende des zweiten Plastochrons; innerhalb eines Plastochrons wird die halbe Zahl der Randzähne angelegt; ihre Ausgliederung schreitet basipetal von oben nach unten fort und wird im fünften bis sechsten Plastochron beendet. Der Endzahn *e* wächst vom dritten bis sechsten Plastochron wie das Gesamtblatt, dann bleibt er zurück und geht als erster Teil der Spreite in den Dauerzustand über. Die Randzähne eins und acht machen zeitlich verspätet denselben Entwicklungsgang durch wie Gesamtblatt und Endzahn; sie schliessen im siebten Plastochron mit geringerer Endlänge ab. Der drittletzte Zahn ($n-3$) ist gegenüber 8 wiederum ein Plastochron verspätet. Randzähne und junge Blattnerven ermöglichen auch Vergleiche zwischen den Teilen der Blattfläche. Diese wachsen gleich schnell; aber an der Spreitenbasis dauert das Wachstum auch nach dem Stadium von Fig. 1 *c* längere Zeit fort. Die Veränderungen in der Spreitenform (Fig. 1 *b*, 1 *c* ausgewachsenes Blatt) beruhen bei Pflanzen nur in geringerem Grade auf Verschiedenheiten der Wachstumsgeschwindigkeiten; sie werden vor allem hervorgebracht durch Verschiedenheiten der Wachstumsdauer. Der Blattstiel *St*, dasjenige verdickte Stück der Blattachse, das keinen Spreitensaum hervorbringt, wird gesondert messbar im dritten Plastochron; er verlängert sich etwas rascher als die Blattspreite und deutlich rascher als das tragende Internodium *I*; das Wachstum erlischt zuerst in Spreite und Stiel erst zuletzt im Internodium. Das Achsenende $VP + I_1 + I_2$, Vegetationspunkt gemeinsam gemessen mit den jüngsten Internodien, verlängert sich gleich rasch wie die ältern Internodien.

Lange schlanke Vegetationskegel wie diejenigen von *Helodea*, *Myriophyllum* und *Hippuris* wachsen rascher in die Länge als die jüngsten Stengelglieder (Askenasy, 1880; Sch. 1923). Ähnlich verhalten sich Endglied und Achsenabschnitte des zusammengesetzten Blattes von *Aralia spinosa* (Nägeli, 1855; Sch. 1933).

(3) Periodischer Formwechsel des Vegetationspunktes

Lathyrus odoratus zeigt zweizeilige Blattstellung und somit eine Symmetrie-Ebene des ganzen Sprosses. Parallel zu dieser Symmetrie-Ebene wurden die Knospen zwischen Daumen und Zeigefinger der linken Hand hindurch mit dem Rasiermesser in ziemlich dicke Schichten gespalten. Durch Aufhellen der mittleren Schnitte ergaben sich klare und vollständige Bilder der Umrisslinien innerhalb der Symmetrie-Ebene. Diese wurden bei vierhundertfacher Vergrößerung gezeichnet und ausgemessen. Die Knospen gelangten in zufälliger Reihenfolge *ABC*... zur Untersuchung; nach Fertigstellung der Zeichnungen ergab sich aus der Gestalt des Vegetationspunktes und der jüngsten Blätter die richtige Reihenfolge *HCEGBDAFI*. Die Umrisslinien von *EBD* und *F* sind in Fig. 3 *a* wiedergegeben. Der Umfang der Zeichnung ist so begrenzt, dass sie das Wachstum und den Formwechsel des Vegetationspunktes im Zeitraume von zwei Plastochron zur Darstellung bringt. Die obersten vier Bilder *E* bis *F* umfassen nur den eigentlichen Vegetationskegel mit dem Scheitelpunkt *S* zwischen der tieferen Blattachsel *A*₃ und

der höheren Blattachsel A_2 . Die rechte Flanke wölbt sich vor zum jüngsten Blatt mit der Spitze B_1 und der Abgrenzung gegen den Vegetationspunkt in der Blattachsel A_1 . Das fünfte Bild stammt wie das erste von der Knospe E ; es ist aber dem Vegetationspunkt das jüngste Blatt hinzugefügt und linke und rechte Flanke vertauscht. Dadurch wird E zur direkten Fortsetzung von F . Im fünften bis achten Bild sehen wir das Blatt E rechts wachsen und sich schärfer vom Vegetationspunkt abgliedern, während links ein neues Blatt B_0 sich vorwölbt. Mit dem untersten Bild kehren wir nochmals zur Knospe E zurück; das Bild des Vegetationspunktes ist

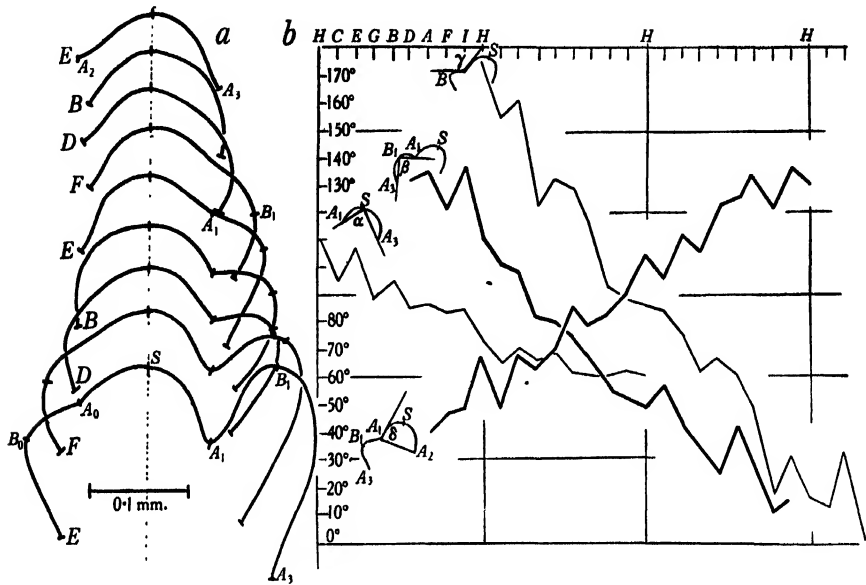


Fig. 3. *Lathyrus odoratus*. (a) Medianschnitte durch 4 Knospen zu einem Bilde des Formwechsels geordnet. S , Scheitel des Vegetationskegels; A_0, A_1, A_2, A_3 , Blattachsen; B_0, B_1 , Blattspitzen. (b) Veränderung von Winkelgrößen. Abscissen: Zeit in Neuntelsplastochron, Reihenfolge der untersuchten Knospen. HCEGBDAFIH. Ordinaten: Winkelgrade. α , Öffnungswinkel des Scheitels; β , Öffnungswinkel der Blattspitze; γ , Blattachsel; δ , Erhebung des Scheitelkegels über seine Basis.

wieder gleich und gleich orientiert wie im Anfang aber mit Hinzufügung je eines Blattes rechts und links.

Den Gesamteindruck können wir so zusammen fassen: "Es ist überall ein beständiges Fortschreiten in der Gliederung der Umrisslinien und der ganzen Oberfläche. Es scheint, dass ein Sprossvegetationspunkt und ein Blattvegetationspunkt, so lange sie ihren meristematischen Charakter bewahren, nicht wachsen können ohne beständig ihre Form zu verändern und sich beständig zu teilen." Der Vegetationspunkt verhält sich einigermassen wie ein Tropfen an einem undichten Wasserhahnen; er schwillt stetig an und gliedert periodisch Teiltropfen ab (Sch. 1916, S. 39).

Wir suchen die Art des Formwechsels genauer zu erfassen, indem wir zuerst einige Winkel messend verfolgen (Fig. 3 b). α sei der Winkel, unter welchem vom Scheitel S aus zwei aufeinander folgende Blattachsen A_1 und A_2 später A_2 und A_3

gesehen werden. Das Stengelende regeneriert sich aus der abgewölbten Scheitelkuppe und spitzt sich im Wachsen zu; der Spitzenwinkel α beträgt da, wo er der Bestimmung zugänglich wird noch 110° und nimmt ab bis auf 60° . Den auf gleiche Art gemessenen Blattspitzenwinkel β sehen wir rascher abnehmen, von 130° auf 10° . Den Winkel γ messen wir zwischen zwei Tangenten an die Blattachsel; im Verlaufe von zwei Plastochron schliesst sich die Blattachsel von 180° auf 0° . Als δ messen wir den Winkel, in welchem sich der Scheitelkegel über die Verbindungslinie der Blattachsen A_1A_2 später A_2A_3 erhebt; er wächst im Verlauf von $2\frac{1}{2}$ Plastochron sehr gleichmässig von 40° auf 140° . Die Masse des Scheitelkegels breitet sich im Hervorwachsen über seine Basis aus und teilt sich in Scheitelkegel

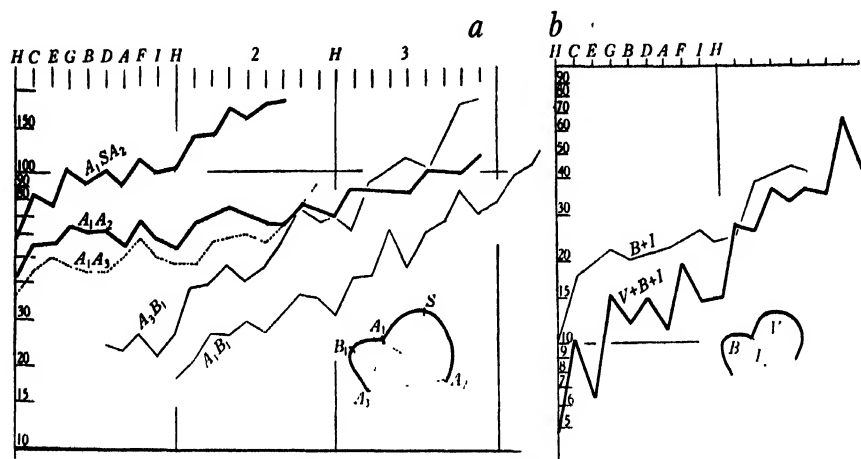


Fig. 4. *Lathyrus odoratus*. (a) Längenmessungen. Abscissen wie in Fig. 3 b. Ordinaten: Logarithmen der Längen der Originalzeichnungen. A_1SA_2 , Scheitelwölbung des Vegetationskegels; A_1A_2 , Basisbreite des Vegetationskegels; A_1A_3 , innere und A_2B_1 , äussere Flanke des Blattes; A_1A_3 , Blattansatz am Stengel. (b) Flächenmessungen. Ordinaten: Logarithmen der Flächen in Einheiten der Originalzeichnungen. $B+I$, Blatt + Internodium; $V+B+I$, Vegetationskegel V übergehend in Vegetationskegel gemeinsam mit dem jüngsten Sprossglied $B+I$.

und junge Blätter. Die Winkelmessungen verstärken den Eindruck, dass der Formwechsel des Vegetationspunktes ein kontinuierlicher Prozess ist, bei dem nur willkürlich ein bestimmter Zeitpunkt als Beginn der Blattbildung herausgegriffen werden kann.

Einige Längenmessungen sind in Fig. 4 a zusammengefasst. Die Basisbreite des Vegetationspunktes A_1A_2 später Stengelbreite A_2A_3 wächst langsam und gleichmässig. Rascher verlängert sich die gekrümmte Scheitellinie A_1SA_2 . Wir erwägen den Gedanken, dass das Aufsteigen der Scheitelwölbung bedingt sein kann durch das Verhältnis zwischen dem Wachstum des Bogens A_1SA_2 und der Sehne A_1A_2 . Die innere Flanke des Blattes A_1B_1 und die äussere Flanke des Blattes A_2B_1 wachsen wiederum rascher als seine Sehne A_1A_3 ; die Blattflanken wachsen auch etwas rascher als die Scheitelwölbung.

Schliesslich gibt Fig. 4 b einige Messungen über die Grösse der Schnittflächen des Vegetationspunktes VP , später des Vegetationspunktes zusammen mit dem

jüngsten Sprossglied $VP + B_1 + I_1$ in mm.^2 der Originalzeichnung, dazu die Kurve für das Sprossglied $B_1 + I_1$ allein. Wir finden einen stetigen Zuwachs der Schnittfläche; dabei ist der Flächenzuwachs für den Vegetationskegel selbst um ein Geringes grösser als für das jüngste Sprossglied.

Ich bin mir bewusst, dass diese spärlichen Angaben zwar den Hinweis auf einen möglichen Weg der Forschung bedeuten, aber noch nicht den umfassenden exakten Nachweis neuer Tatsachen. Auch möchten wir neben dem Wachstum der Umrisslinien das Wachstum der Oberflächen und neben dem Wachstum der Schnittflächen das Wachstum der Volumen kennen. A. Schmidt (1924, S. 365) hat gezeigt, wie sich für flache Scheitel in der Scheitelansicht das stetige Wachstum von einer Minimalfläche zu einer Maximalfläche vor der Blattbildung verfolgen lässt. Eine weitere Möglichkeit für Wachstumsbestimmungen innerhalb des Meristems ist die Auszählung der Kernteilungsfiguren (Sch. 1923, S. 58). Als vorläufiges Resultat ergab sich gleiche Häufigkeit der Teilungen in den Oberflächenschichten und im Kern des Vegetationskegels. Die Prozentzahl der Kernteilungsfiguren unter der Gesamtzahl der Kerne war bei *Lathyrus latifolius* im Dermatogen 13.23 ± 1.08 , im Periblem 15.21 , im Plerom 16.02 . Eine frühere Untersuchung hatte ergeben im Dermatogen 8.49 , im Plerom 8.14 .

(4) Wachstumsbewegung im Vegetationspunkt

Fig. 5 b gibt ein Gesamtbild der innern Wachstumsbewegung im Vegetationspunkt und den jüngsten Sprossgliedern von *Lathyrus latifolius*. Beim Wachsen der Knospe wird der Scheitel S in der Richtung der Achse vorwärts geschoben. Um das Wachstum zu veranschaulichen denken wir den Punkt S als Initialpunkt, als Quellpunkt, aus dem die ganze Sprossoberfläche im Wachsen herausfließt, im Raume festgehalten. SZ ist dann die kurze Initiallinie, aus welcher heraus die Gesamtmasse der Sprosse immer wieder regeneriert wird. Für jede Schar entsprechender Punkte Blattspitzen, Blattachseln u.s.f. ergeben sich bei zweizeiliger Blattstellung zwei symmetrische Verschiebungskurven aa , bb , cc , ... als Wege die bei der Wachstumsbewegung durchlaufen werden. Auf den Verschiebungskurven sind eine Reihe von Punkten hervorgehoben als Stationen, welche von den Wanderpunkten im Zeitabstand eines halben Plastochrons passiert werden. Punkte im Zeitabstand von einem Plastochron sind gleichzeitig von verschieden alten Gliedern besetzt; sie liegen alternierend links und rechts auf den gleichnamigen Verschiebungskurven z. B. B_0 , B_1 , B_2 , B_3 , B_4 auf bb .

Vom Scheitelpunkt S aus verschieben sich zunächst alle Punkte längs der Oberfläche und wandern dabei auf die Flanken der Scheitelkuppe; von dort aus zerstreuen sie sich zwischen den Kurven b für die Blattspitzen und a für die Blattachseln. Der Scheitelkegel ist geschichtet; zwischen S und Z laufen zuerst alle Verschiebungskurven periklin, parallel zur Oberfläche. Weil SZ mehrere Zeldurchmesser lang ist, entstehen getrennte Zellschichten, Dermatogen und erste und zweite Periblemschicht oder eine Tunica aus drei Schichten (Schmidt, 1924) von Z aus divergieren die Kurven e , f und m ; von Z aus ergänzt sich das Plerom oder der Korpus.

Die Wachstumsbewegung von *SZ* aus ist zuerst gleichförmig; sie wird aber bald zu einer Wellenbewegung mit wachsenden Ausschlägen. Am stärksten ist die Bewegung der Oberfläche; sie setzt sich tief ins Innere fort. Die Punkte an der Grenze der zweiten und dritten Tunicaschicht zerstreuen sich zwischen die Kurven *c* der Mittelrippen und *d* der Blattachsen. Selbst im Stengelmark wechselt noch längs der Achse *m* die Richtung der Zellreihen. Vielleicht besteht doch eine Ähnlichkeit mit den Wasserwellen darin, dass sie an der Oberfläche erregt werden und gegen die Tiefe ausklingen.

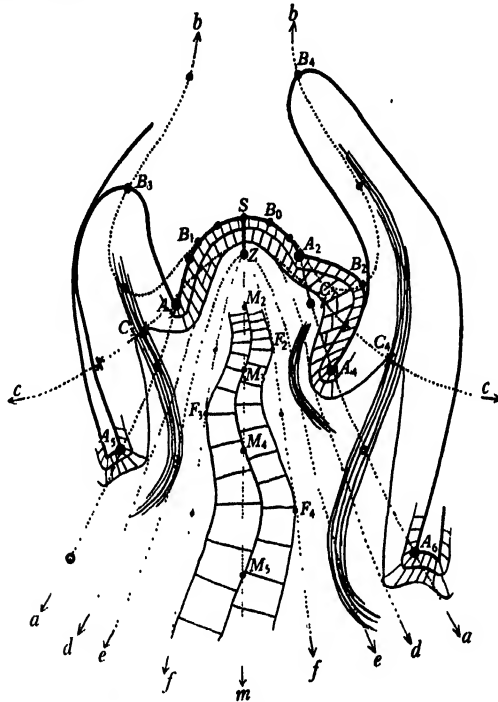


Fig. 5. Wachstumsbewegung in der Knospe von *Lathyrus*; Schema. *S*, Scheitel; *SZ*, Initialstrecke; *B*₀ bis *B*₄, Bewegung der Blattspitzen auf den Verschiebungskurven *b*; *A*₁ bis *A*₄, Bewegung der Blattachsen auf den Verschiebungskurven *a*; *C*, Punkte der Blattachse innerhalb der zweiten Tunicaschicht; *F*, Punkte an der Aussengrenze des Marks; *M*, Punkte der Markmitte.

Im Zellnetz des Scheitelkegels unterscheiden wir die periklinen Wände parallel zur Oberfläche, die im Laufe der allgemeinen Wachstumsbewegung stärkstes Flächenwachstum erfahren, und die antiklinen Wände senkrecht zur Oberfläche, die nicht wachsen und dafür bei den Zellteilungen durch Neubildung an Zahl vermehrt werden. Diese Wachstumsweise des Scheitels der Angiospermen macht die Existenz von Periklinalchimären möglich; uns interessiert sie hier viel mehr in anderer Richtung, indem wir nach den Zusammenhängen fragen, welche bestehen zwischen dem periodischen Formwechsel des Vegetationspunktes und seiner innern Wachstumsbewegung.

Allgemein wird angenommen, dass die Blattbildung hervorgerufen werde durch eine lokalisierte Zunahme der Zellteilungsintensität in den tieferen Schichten der

Tunica. Wir prüfen diese scheinbar so selbstverständliche aber noch nie bewiesene Annahme an Hand der Fig. 6 a. Sachs (1878, 1879) hatte vorausgesetzt, dass die am Scheitel von ihm wahrgenommene rechtwinklige Schneidung der Periklinen und Antiklinen auch bei der Blattbildung erhalten bleibe. Danach müssten die Antiklinen 1-2 des Blattes B_0 beim Hervorwölben des Blattes zum Stand von B_1 ausserordentlich stark divergieren. Wenn unter dem werdenden Blatt sowohl die

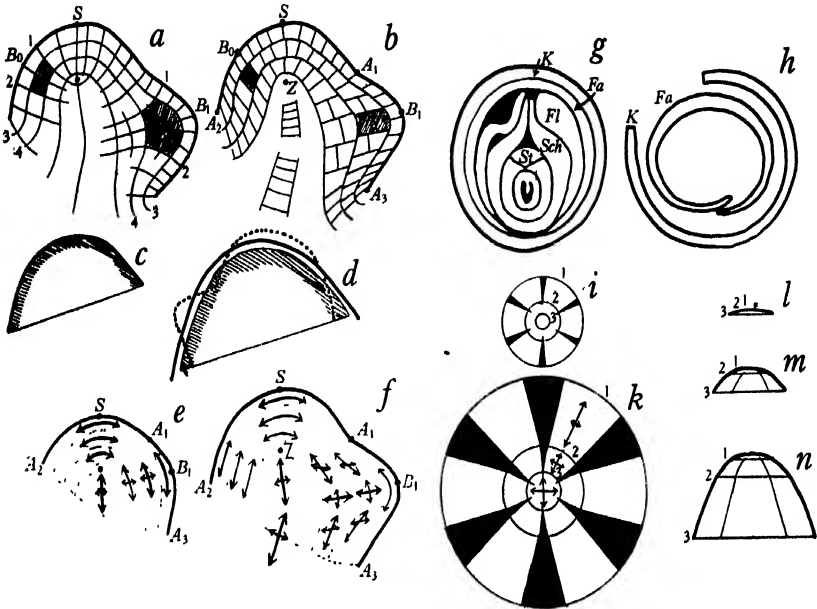


Fig. 6. (a) Schema der Wachstumsbewegung bei der Blattanlage (Periblem schraffiert) nach dem Prinzip der rechtwinkligen Schneidung. (b) Schiefstellung der antiklinen Wände bei der Aufwölbung des Vegetationskegels zwischen A_2 und B_0 und bei der Faltung des jungen Blattwalls zwischen A_1 , A_2 und B_1 . (c) und (d) Beziehungen zwischen Oberflächenwachstum, Volumenwachstum und Faltung der Oberfläche. (e) und (f) Wachstumsrichtungen in Scheitel und Blattanlage. Einfache Doppelpfeile bedeuten ausschliessliches Wachstum parallel zum Pfeil; gekreuzte Doppelpfeile veranschaulichen die Verteilung des Wachstums auf verschiedene Richtungen. (g) *Lathyrus vernus*. Querschnitt einer Blütenknospe. K, Kelch; Fa, Fahne; Fl, Flügel; Sch, Schiffchen. St, Staubfäden. (h) Kelch und Fahne aus dem vorigen Querschnitt herausgelöst zeigen Spannungen in der Knospe an. (i) und (k) Schema des Wachstums einer mit Hilfe radialer Schnitte in die Ebene gelegten Scheitelfläche. 1, 2 und 3 wachsende Kreise. Die gekreuzten Pfeile in k deuten auf gleichmässiges Wachstum im Zentrum und auf vorwiegend radiales Wachstum an der Peripherie. (l), (m) und (n) Seitenansichten zu i und k nach Schliessung der radialen Lücken.

Periklinen 3-4 als auch die Antiklinen 1-2 so stark auseinander treten, so ergibt sich in der Tat für den schraffierten Komplex ein Wachstum, das die Intensität des Wachstums der Nachbargebiete weit übertrifft. Eine entsprechende Häufung von Kernteilungsfiguren habe ich nicht gesehen.

Auch das Studium des Zellnetzes weist nach anderer Richtung hin (Fig. 6 b). An der Scheiteltuppe sind die Zellumrisse rechtwinklig, ebenso in nächster Nähe der Blattspitze B_1 . An den Seiten der Blattachsen A_1 , A_2 , A_3 werden die Rechtecke zu ausgeprägt schiefwinkligen Rhomboiden verschoben. Dies geschieht im Zusammenhang mit der Faltung der Schichten und kann nachgeahmt werden, indem man

auf den Schnitt eines Buches Querlinien zeichnet und dann ein Paket Blätter faltet. Die periklinen Schichten falten sich, indem sie ihr vorwiegendes periklines Flächenwachstum aufrecht erhalten; an den Orten wo sich im Dermatogen eine Blattfalte erhebt, wird in den innern Tunicaschichten das Flächenwachstum durch antiklines Dickenwachstum ersetzt.

Die Ursache des Formwechsels kann statt in örtlich lokalisierten Wachstumserscheinungen auch im Zusammenwirken der vereinigten Schichten gesucht werden. In dieser Hinsicht ist zu fragen nach der Beziehung des Oberflächenwachstums zum Volumenwachstum (Sch. 1917). Theoretisch sind drei Fälle zu unterscheiden. Es kann ein überwiegendes Oberflächenwachstum einen Formwechsel erzwingen mit der Richtung nach zunehmender Gliederung; es können Oberflächenwachstum und Volumenwachstum im Gleichgewicht stehen, sodass Wachstum ohne Formwechsel möglich ist; es kann ein Überwiegen des Volumenwachstums einen Formwechsel erzwingen mit der Richtung nach Vereinfachung der Form. Als Beispiel für den ersten Fall denken wir an die Sprossbildung, als Beispiel für den dritten Fall an Früchte, die im Wachsen der Kugelform zustreben. Gleichgewicht herrscht, wenn der Wachstumskoeffizient für die Längen α derjenige für die Flächen β und derjenige für die Volumen γ in der Beziehung stehen $\alpha^2 = \beta$; $\alpha^3 = \gamma$. Ist beim Spross, wie nach den Kernteilungszählungen wahrscheinlich, $\beta = \gamma$ oder annähernd gleich γ , so überwiegt das Oberflächenwachstum.

Lassen wir in Fig. 6 c die Längen auf das 1.41fache wachsen, so wächst die Fläche auf das Doppelte (6 d äussere Umrisslinie); lassen wir die Längen auf das 1.26fache wachsen, so wächst das Volumen auf das Doppelte (6 d schraffierte Fläche). Wächst die Oberfläche in gleichem Mass wie das Volumen, so wird die Oberfläche zu gross; durch Faltung der Oberfläche und Anpassung des Kerns (punktierte Linie) kann der Zusammenhang erhalten bleiben.

Die Fig. 6 e und f veranschaulichen die Wachstumsrichtungen im Scheitelkegel und im jungen Blatt. Einfache Doppelpfeile bedeuten ausschliessliches Wachstum in perikliner Richtung; die kreuzweis vereinigten Doppelpfeile bedeuten gleichzeitiges Wachstum in perikliner und antikliner Richtung, wobei die längeren Arme des Kreuzes auf die vorherrschende Wachstumsrichtung hinweisen. Im Stengelmark herrscht deutlich das Wachstum in der Richtung der Längsachse vor; die Längsreihen von Zellen dringen bis nahe unter den Zentralpunkt Z vor. Im Zusammenwirken des periklinen Wachstums der Tunica oberhalb Z mit dem Längenwachstum des Markes unterhalb Z liegt eine weitere mögliche Ursache für den Verlauf des Formwechsels; die Wölbung des Vegetationskegels wächst rascher als seine Basisbreite.

Ein Zusammenwirken von Teilen mit verschiedenartiger Wachstumstendenz zu einem harmonischen Formwechsel setzt gegenseitige Anpassung der Teile voraus, vermittelt durch Zug- und Druckkräfte und durch Reaktion des Wachstums auf diese Kräfte (Sch. 1911, S. 23). Die Verschiebung rechtwinkliger Zellumrisse zu schiefwinkligen war bereits ein Hinweis auf diese Kraftwirkungen; ich habe auch einmal den Versuch gemacht, dieselben direkt nachzuweisen. Die Hälften längs gespaltener Vegetationskegel krümmen sich gegen die Achse ein (Sch. 1918). Zwei

Fehlerquellen liegen aber vor. An der Spaltfläche zeigen die Zellen starke Krampfpasmolyse; es ist damit zu rechnen, dass dadurch ein Zug auf die Zellwände hervorgerufen wird, der im intakten Zustande fehlte. Die starke Krümmungstendenz der jungen Blätter kann auf die angrenzenden Rindenteile einwirken und aktive Krümmung derselben vortäuschen (Schneider, 1926).

So müssen wir uns für einmal damit begnügen aus den deutlichen Reaktionen jugendlicher Blätter (Sch. 1919) einen Hinweis zu entnehmen auf Reaktionen, die möglicherweise auch am Scheitelkegel wirksam sind. In der Blütenknospe von *Lathyrus vernus* (Fig. 6 g) falten sich die inneren Kronblätter, Flügel und Schiffchen aus Raummangel. (Vom Studium dieser Faltungen aus, die zu charakteristischen Bildungen an der ausgewachsenen Schmetterlingsblüte führen, haben meine Studien am Vegetationskegel ihre bestimmte Richtung genommen (Sch. 1911).) Löst man bei einem dicken auf Wasser schwimmenden Schnitt die Blütenteile auseinander, öffnet man dabei auch an einer Stelle den Kelchring, so zeigen sich sofort charakteristische Reaktionen. Der Kelchring klappt auseinander durch Abnahme der Krümmung; die Fahnenränder schieben sich übereinander durch Zunehmen der Krümmung. Die Reaktion wechselt mit dem Alter. Der junge Kelch ist hypoplastisch gespannt, später wird er epinastisch. Die den Spannungen entsprechenden Bewegungen sind beim Kelch durch seine Röhrenform verhindert. Bei anderen Teilen finden die Bewegungen mehr oder weniger gehemmt ihren Ausdruck in hyponastischer Krümmung gegen den Vegetationskegel und in epinastischer Blattentfaltung aus der Knospe heraus. Ich vermute, dass diese Spannungen und Bewegungen nicht bloss auf Wachstumsunterschiede zurückzuführen sind sondern im Feinbau der Membranen ihre Ursache haben. Bestehen solche Krümmungstendenzen auch in den Tunicaschichten des Vegetationskegels, so können sie für Form und relative Grösse der Blattanlagen mitbestimmend wirken.

Die Bestimmung einer körperlichen Form kann geschehen erstens durch Gegeneinanderwirken der Wachstumstendenzen verschiedener Schichten; sie kann bestimmt sein zweitens durch Krümmungstendenzen der Oberflächenschichten; sie kann bestimmt sein drittens durch die Verteilung des Wachstums innerhalb der Oberflächenschicht. Fig. 6 i und k seien im Schema Stücke einer Scheitelfläche, durch Einschnitte am Rande in eine Ebene ausgebreitet. Die konzentrischen Kreise 1, 2 und 3 sind im Wachstum begriffen. Das Feld innerhalb 1 wächst gleichmässig nach allen Richtungen; die sechs Teilfelder zwischen 1 und 2 wachsen radial stärker als tangential, sodass zwischen ihnen Lücken entstehen. Die trapezförmigen Stücke zwischen 2 und 3 zeigen noch ausgeprägteres Vorherrschen des radialen Wachstums gegenüber dem tangentialen Wachstum; die Lücken verbreitern sich. Schneidet man die Zeichnungen aus und schliesst man die Lücken, so ergibt sich der räumliche Formwechsel Fig. 6 l, m, n. Man kann oft sehen, wie um ein Wachstumszentrum S des Vegetationspunktes herum die Zellen in Radialreihen sich ordnen. Es bleibt zunächst fraglich, ob das geschieht in Anpassung an eine andersartig verursachte Aufwölbung des Scheitels, oder ob diese Wachstumsanordnung Ursache der Aufwölbung ist. Das Prinzip einer räumlichen Formbildung durch Wachstumsverteilung innerhalb einer Fläche kommt auch in Frage für

Formbildungen innerhalb einer Zelle, z. B. das Auswachsen von Haaren; hier ist es denkbar, dass ohne wesentliche Einwirkung des Volumeninhaltes protoplasmatischer Wandbelag und Wand allein die Form bestimmen (vergl. Sch. 1916, S. 64; Nägeli, 1855; bei Sch. 1933, S. 247). Wir sind heute nicht in der Lage zu entscheiden, welcher Art die formbildenden Kräfte am Vegetationspunkte seien. Wohl aber sehen wir eine ganze Reihe von Möglichkeiten, deren Erwägung die Forscher zu bestimmten Fragestellungen und Untersuchungen anregen kann.

(5) *Innere und äussere Differenzierung*

Im Gewebe des Vegetationskegels müssen wir rechnen mit einem Zusammenwirken verschiedenartiger Wachstumstendenzen; wir werden diese Wachstumstendenzen klarer erkennen, da wo sie sich beim Aufbau bestimmter Organe einzeln manifestieren können. Wir unterscheiden zweckmässig zwischen drei hauptsächlichen Wachstumsformen der Meristeme (Sch. 1926, S. 16). Gleiches Wachstum nach den drei Hauptrichtungen des Raumes zeigen die massigen Meristeme der Sporangien. Vorwiegendes Flächenwachstum zeigen die Plattenmeristeme der Blattspreiten, vorwiegendes Längenwachstum zeigen die Rippenmeristeme der Blattstiele und der Stengel; besonders scharf ausgeprägt ist die isokline Zellteilung im Rippenmeristem der jungen Wurzeln (Giesenhausen, 1905).

Fig. 7 *a* zeigt den Querschnitt durch das Rippenmeristem einer jungen Blatt-*ranke* von *Lathyrus latifolius*. Innerhalb des Dermatogens herrscht keine bestimmte einfache Anordnung der Zellen; die Kernteilungen, deren Spindelfigur in die Bildebene fällt, ergeben teils tangentielle teils radiale Teilungswände. Im Längsschnitt Fig. 7 *b* und in der Oberflächenansicht des Dermatogens Fig. 7 *c* treten deutliche Längsreihen von Zellen hervor. Die Kernspindeln stellen sich meistens in die Längsrichtung des Organs ein; die Teilungswände laufen quer. Weil aber immer wieder Längswände eingeschaltet werden, erreichen die Zellreihen nur beschränkte Länge.

Die Reihe der Fig. 7 *h* bis *m* zeigt bei Abnehmen der Vergrösserung gezeichnet Querschnitte durch das Fiederblatt von *Lathyrus latifolius*; bei sehr geringem Dickenwachstum finden wir ein lange andauerndes starkes Flächenwachstum. Am Fiederrand zeigt sich eine schwache Häufung der Kernteilungsfiguren (Fig. 7 *d*). In der Nähe des Randes wechselt die Teilungsrichtung; aus der Reihe der Randzellen des Periblems entstehen dabei drei bis fünf Zellschichten (Noack, 1922); diese dehnen sich aus mit ausschliesslicher Teilung senkrecht zur Fläche. Der Fiederlängsschnitt (Fig. 7 *f*) zeigt genau wie der Querschnitt kurze Zellreihen parallel zur Oberfläche. Im Gegensatz dazu zeigen die Oberflächenansicht (7 *e*) und der Flächenschnitt (7 *g*) wechselnde Teilungsrichtung und gleichmässige Ausdehnung der Fläche nach allen Richtungen.

Der Vergleich der Rippen- und der Plattenmeristeme macht klar, dass Stengel und Blattspreite, die beiden Grundformen des Pflanzenkörpers herzuleiten sind aus den einfachen Wachstumseigenschaften ihrer Meristeme. Und diese Wachstumseigenschaften, die sichtbar werden in der vorherrschenden Orientierung der Kernteilungsfiguren, sind wiederum ein Hinweis auf eine nicht direkt wahrnehmbare

Anisotropie des Protoplasmas. Eine gesetzmässige Anordnung der Protoplasma-moleküle, namentlich der Moleküle des protoplasmatischen Wandbelages, erscheint als zunächst letzte denkbare Ursache der Wachstumsanordnung und der Formbildung.

Aus dem Rippenmeristem oder aus dem Plattenmeristem werden in strangförmiger Anordnung langgestreckte Prokambiumzellen herausgeschnitten. Solche sind in Fig. 7 *a* und *d* quer, in Fig. 7 *f* längs geschnitten.

Wir betrachten den Zusammenhang der Meristemarten nach ihrer Herkunft aus dem Urmeristem des Vegetationskegels. Dieses hat in der Tunica (zwischen *S*

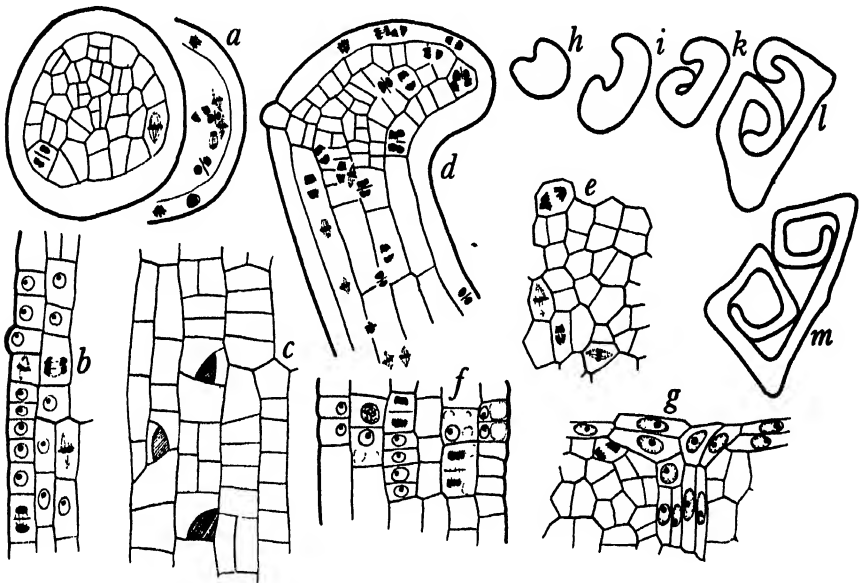
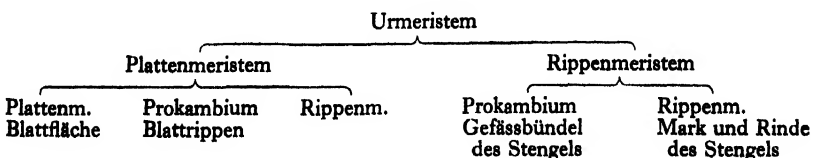


Fig. 7. *Lathyrus latifolius*. (a) Querschnitt durch das Rippenmeristem einer jungen Ranke, rechts Lage der Kernteilungsfiguren aus mehreren Parallelschnitten. (b) Längsschnitt einer jungen Ranke. (c) Flächenansicht des Dermatogens einer jungen Ranke, Spaltöffnungsmutterzellen schraffiert. (d) Querschnitt durch Randmeristem und Plattenmeristem eines jungen Fiederblattes, Kernteilungsfiguren aus einer Reihe von Parallelschnitten in eine Zeichnung übertragen. (e) Dermatogen eines Fiederblattes im Flächenschnitt. (f) Fiederblatt im Längsschnitt. (g) Mesophyll im Flächenschnitt, Entstehung von Prokambiumsträngen. (h) bis (m) Entwicklung des Querschnitts beim Fiederblatt, in abnehmender Vergrößerung gezeichnet.

und Z Fig. 5) den Charakter eines Plattenmeristems, im Korpus (um Z) den Charakter eines massigen Meristems.



Wir verstehen aber die Wege der Differenzierung und Umdifferenzierung nicht, wenn wir in erster Linie auf die Abstammung der Gewebe achten. Wir verstehen

sie besser beim Blick auf die Lage der Zellen in den jüngsten Blattanlagen und den angrenzenden Stengelteilen, den Blattsockeln ("soubasements foliaires", Louis, 1935). Am Vegetationskegel eilt die äussere Differenzierung, der Formwechsel der Oberfläche, der inneren Differenzierung, der Umbildung der Zellinhalte voraus und bestimmt sie. Die Verteilung von Plattenmeristem und Rippenmeristem, später die Verteilung der Prokambiumstränge wird bestimmt durch die Lage zum jungen Blatthöcker. Die dünneren Seitenteile des Blattwalles werden zu Plattenmeristem; der dicke Mittelzapfen des Blattwalles wird Rippenmeristem; inmitten der Mittelrippe und ihrer Fortsetzung im Blattsockel sondert sich der erste Prokambiumstrang aus, die Blattspur (Fig. 5). Von der Stengelbasis aus, aber auch von den Blattspitzen aus schreitet die Umwandlung des Teilungsgewebes in Streckungsgewebe und Dauergewebe fort. Ohne Kenntnis der Faktoren, welche die Zellen in dieser bestimmten Weise auf ihre Lage reagieren lässt, und ohne uns für oder gegen bestimmte Hypothesen über diese Faktoren zu entscheiden (Priestley, 1929) sind wir doch durch das Gesamtbild der wachsenden Knospe gezwungen, das Blatt mit den tragenden Teilen des Stengels als wachstumsphysiologische Einheit zu betrachten. Diese Einheit ist in der Morphologie bekannt als Sprossglied oder Phytom.

II. BLATTSTELLUNG

(1) *Phytonismus*

Der Phytomismus (vergl. Schoute, 1931) entstammt der formalen Morphologie, der vergleichenden Betrachtung ausgewachsener Pflanzenteile. An diesen tritt vor allem der Gegensatz zwischen den ausdauernden Sprossachsen und den kurzlebigen, hinfälligen Blättern hervor; aber die Aufmerksamkeit der Morphologen wurde auch auf die Zusammengehörigkeit des Blattes mit dem tragenden Stengelteil gelenkt. Beide zusammen bilden die Einheit eines Phytom. Diese Einheit wird umso deutlicher je mehr wir die Jugendzustände des Sprosses betrachten. Ein Phytom ist eine Wachstumseinheit (Priestley & Scott, 1933)¹; Blatt und Blattsockel, Blatt und tragendes Stengelglied sind eine Wachstums- und Entwicklungseinheit von ihrer Ausgliederung aus dem Vegetationskegel ab. Phytomismus ist die theoretische Form, in welcher die formale Morphologie die Periodizität der Formbildung am Vegetationspunkt erfasst und zum gedanklichen Ausdruck gebracht hat.

Im Hinblick auf unsere Kenntnis der ontogenetischen Herkunft der Phytomen werden wir uns dieser Lehre in freier Form bedienen und namentlich auf scharfe Abgrenzung der Phytomen gegeneinander keinen Wert legen. Jeder Phytom wird in seiner Ausbildung beherrscht vom frei vorragenden Blatt; dieses wirkt auf den Stengel ein; so weit seine Einflussphäre reicht, so weit reicht sein Phytom.

Der Spross ist ein Ganzes, das sich aus der undifferenzierten Masse des Vegetationskegels fortbildet. Die Phytomen werden periodisch nacheinander und zum Teil auch nebeneinander aus dem Urmeristem abgegliedert. Die Kette der Phytomen endet im Vegetationspunkt und wird aus diesem heraus verlängert (Sch. 1934, S. 65).

¹ *Biological Reviews*.

(2) *Phylogenie der phytonischen Sprossgliederung*

Gesetzmässige Blattstellung ist verknüpft mit dem Vereint-Wachsen des Vegetationspunktes und der jungen Phytomen innerhalb der grösseren Wachstumseinheit der Sprossknospe. Dieses Vereint-Wachsen gehört einer höheren Stufe der Formbildung an. Phylogenetisch ist es von einem getrennten Wachstum der einzelnen Glieder herzuleiten. Gliederung in Phytomen ist nicht die ursprüngliche sondern die abgeleitete hochdifferenzierte Form des Sprosswachstums (Sch. 1936, S. 563). Die Gliederung des Sprosses in Phytomen ist auch verknüpft mit der Bildung einer ausgedehnten Wachstumszone; sie tritt auf, wo an ein vielzelliges Urmeristem

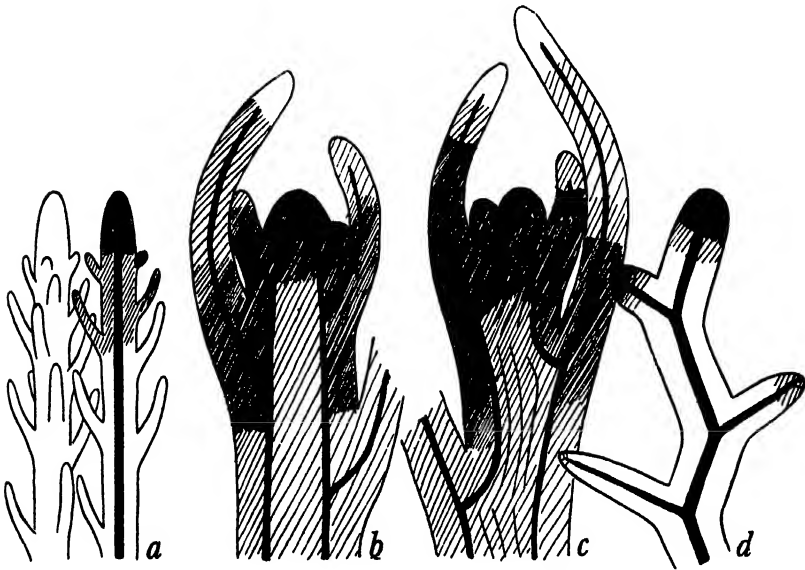


Fig. 8. Ableitung des Phytomismus. Scheitelzellen und Initialzellen sowie Gefässbündelstränge schwarz, Teilungsgewebe dicht schraffiert, Streckungsgewebe locker schraffiert, Dauergewebe weiss. (a) Microphyller Typus ohne Blattspuren. (b) Microphyller Typus mit Blattspuren. (c) Macrophyller Typus. (d) Dichotomer Thallus.

eine ausgedehnte Teilungszone mit Plattenmeristem, Rippenmeristem und Prokambium anschliesst.

Zwei Wege der phylogenetischen Herleitung der Phytomen sind denkbar; der eine führt von zerstreuten Anhangsgebilden zum mikrophyllen Typus, der andere von gabeliger Verzweigung zum makrophyllen Typus.

Wir denken uns (Fig. 8 a) als Ausgangsform einen Spross mit Spitzenwachstum, vermittelt durch ein Bildungsgewebe von beschränktem Umfang mit Scheitelzelle oder einer Anzahl von Initialzellen. Die Gewebedifferenzierung, insbesondere auch die Ausbildung der Stele schreitet von unten nach oben, rein akropetal fort. Hinter der Teilungszone (dicht schraffiert) folgt eine kurze Streckungszone (locker schraffiert). Aus oberflächlichen Zellen oder kleinen Zellgruppen wachsen durch lokale Erneuerung des Teilungswachstums kleine Anhangsgebilde

aus, die als Haare, Schuppen oder Emergenzen bezeichnet werden können. Sie entstehen unabhängig voneinander und darum in unregelmässig zerstreuter Stellung. Sie entstehen über schon weit ausgereiften Partien der Rinde und sind darum ohne Verbindung mit der Stele.

Der Fortschritt zum beblätterten Spross geschieht durch Ausdehnung der Teilungszone, und durch Anlage der Seitenglieder innerhalb der Basis der Teilungszone. Die Anhangsgebilde, die wir jetzt Blätter nennen, treten durch das gemeinsame Wachstum in Beziehung zueinander und ordnen sich nach bestimmten Stellungsgesetzen. Die Blätter stehen mit den tragenden Rindenteilen zugleich im Teilungswachstum; dies bedingt die Anlage von Blattspursträngen mit Anschluss an die Stele. Frühzeitig angelegte Blätter eilen im Wachstum voraus; die Gewebedifferenzierung beginnt an der Spitze der Blätter und schreitet in denselben basalwärts fort (Fig. 8 *b*). Die Blattspurbündel eilen in der Ausbildung der stamm-eigenen Stele voraus. Die Blattbildung wirkt stark auf die Rindenbildung ein; der Stengel ist von den Blattbasen berindet; die Stele des Stengels wird zum Verschmelzungsprodukt der Blattspurbündel. Blätter und Blattsockel bilden als Phytomen die eigentlichen Bausteine des Sprosses, die den zentralen Markstrang umkleiden (Perikaulom nach Potonié).

Als zweite Ausgangsform denken wir uns einen dichotom verzweigten Thallus (Fig. 8 *d*). Die Zweige wachsen wie in Fig. 6 *a* mit einem Bildungsgewebe von beschränktem Umfang. Die Gabelung wird eingeleitet durch eine Halbierung der Scheitelzelle oder durch Neubildung einer Scheitelzelle in einem jungen Segment; der Verdoppelung der Scheitelzelle folgt bald eine Trennung des Teilungsgewebes und des Streckungsgewebes. Der stärkere Gabelast, die Sprossachse und der schwächere Gabelast, das Blatt wachsen aus mit getrenntem Bildungsgewebe. Blätter sind nach dieser Ableitung Kurztriebe oder Telome mit begrenzter Wachstumsdauer (Zimmermann, 1935).

Der Fortschritt geschieht dadurch, dass bei ausgedehnterem Bildungsgewebe die Verzweigungen rascher aufeinander folgen. Das Bildungsgewebe umfasst dann zugleich mit dem Ende der Sprossachse auch die Anlagen von einem, zwei oder mehreren Blättern; ferner ist eine ganze Anzahl von Blättern und Internodien der Sprossachse zugleich im Streckungswachstum begriffen. Die Differenzierung zwischen Sprossspitzen und Blattspitzen verstärkt sich; die Sprossspitze behält ihr Wachstumszentrum; die Blattanlagen entstehen seitlich. Die Blätter eilen in Wachstum und Gewebeausbildung der Sprossachse voraus. In der Blattspur kehrt sich die Richtung der Differenzierung um. Zuerst bilden sich die äusseren Teile aus; die Blattspur dringt basipetal aus dem Blattgrund in die weniger differenzierten Stengelteile vor. Die Stele des Sprosses baut sich aus den Blattspuren auf; der Spross erscheint als eine Kette aneinander gereihter Phytomen.

Die Blätter mit den tragenden Rindenpartien und mit den Blattspuren oder die Phytomen sind Einheiten, die sich aus dem Urmeristem herausbilden. Sie ordnen sich bei ihrer Entstehung und sind auch im Augenblicke der Entstehung als verschiebbar zu betrachten. Welche der möglichen Anordnungen verwirklicht wird hängt von der Symmetrie und von der relativen Grösse der Blattanlagen ab.

(3) Konstruktion von Blattstellungsschemata

Um von der entwicklungsgeschichtlichen Beschreibung zu einer geometrischen Theorie der Blattstellung überzugehen greifen wir zurück auf die Übersicht des Formwechsels in Fig. 5. Wir konstruieren nach den gleichen Grundsätzen ein Schema für eine Spiralstellung in Fig. 9. Fig. 9 *a* zeigt Wachstum und Verschiebung eines Blattes vom Scheitelpunkt *S* hinweg. Die Blattspitze wandert auf der Verschiebungskurve *B*₁ bis *B*₅ nach aussen und oben; die Punkte *C*₁ bis *C*₅ an der Grenze von Blatt und Blattsockel bewegen sich nach aussen; die Blattachselpunkte

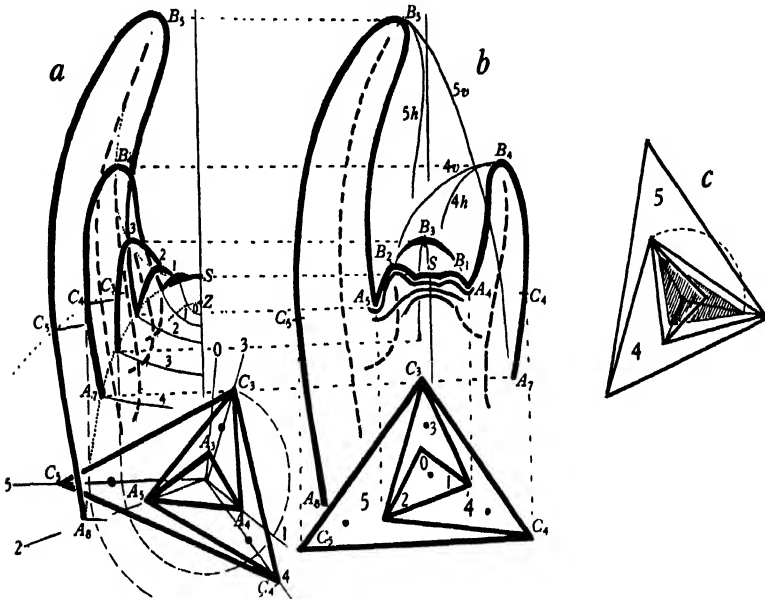


Fig. 9. Schemata für die Wachstumsbewegung bei Spiralstellung der Blätter. (a) Im Aufriss alle Blätter nach derselben Flanke gedreht. *B*₁ bis *B*₅, Blattspitzen; *A*₁ bis *A*₅, Blattachsels; *C*₁ bis *C*₅, äussere Ecken der Blattsockel. Im Grundriss Verteilung der Punkte *C* und *A* auf die verschiedenen Flanken nach der Divergenz von 125° . (b) Verteilung der Blattaufrisse auf die verschiedenen Flanken *4v* und *4h*, *5v* und *5h* sind die vorderen und hinteren Blattränder der Blätter 4 und 5. (c) Die schraffierte Minimalfläche des Scheitels wächst heran zu einer Maximalfläche und lässt nach Abgliederung des jüngsten Blattes 3 wieder eine Minimalfläche zurück.

*A*₁ bis *A*₅ verschieben sich längs der idealen Stengeloberfläche. Jeder Blattsockel hat die Höhe von drei Internodien; die innere Blattachsel von Blatt 5 liegt so hoch wie die äussere Blattachsel von Blatt 2. Die Lage der Prokambiumstränge ist durch gestrichelte Linien angegeben; sie liegen alle ausserhalb einer von *Z* ausgehenden Verschiebungskurve. Von Blatt 1 zu Blatt 3 verfolgen wir die Aufrichtung der Aussenkante des Blattes; bei Blatt 4 und 5 wird diese Kante nach aussen überhängend. Einer Höhe des Blattsockels gleich drei Internodien entspricht eine Annäherung an die $1/3$ Stellung. Im Grundriss (Fig. 9 *a* unten) ist deshalb eine Divergenz von 125° angenommen. Gezeichnet sind für die äusseren Blätter die Punkte *C*₁ bis *C*₅ aussen und *A*₁ bis *A*₅ innen an der Ansatzfläche. Die Punkte

A_5 bis A_3 sind zugleich äussere Grenzpunkte der Blätter 2 bis 0. Punkt C_5 des Grundrisses liegt senkrecht unter Punkt C_5 des Aufrisses. Punkt C_4 ist im Grundriss um einfache Divergenz gedreht, Punkt C_3 um zweifache Divergenz u.s.w. Durch gerade Verbindungslinien wird dargestellt, wie jedes Blatt die innern Teile der Knospe auf zwei von drei Seiten einschliesst.

In Fig. 9 *b* ist unten der Grundriss wiederholt mit einer leichten Drehung der ganzen Figur. Senkrecht darüber ist der Aufriss gezeichnet mit richtiger Verteilung der Punkte nach den verschiedenen Radien; die Höhe der Punkte stimmt mit Fig. 9 *a* überein. Das anschauliche Bild der Sprossspitze, das so entsteht, ist ergänzt durch die vorderen und hinteren Blattränder. $5v$ und $4v$ überschneiden sich auf der Vorderseite; $5h$ und $4h$ verschwinden hinter Blatt 3 auf der Hinterseite.

Fig. 9 *c* veranschaulicht das Wachstum der Scheitelfläche. Nach Abgliederung der Blätter 5, 4 und 3 ist eine Scheitelfläche 2, 1, 0 zurückgeblieben. Es ist eine Minimalfläche (Schmidt, 1924). Drehen wir diese Minimalfläche um den Divergenzwinkel zurück in die schraffierten Lage, so sehen wir wie durch allseitiges Wachstum unter geringer Formveränderung eine Maximalfläche entsteht, von welcher ein jüngstes Blatt 3 abgeschnitten wird; dadurch bleibt wieder eine Minimalfläche zurück.

Längsschnitte und Längsansichten der Knospen zeigen einen starken Formwechsel der Phytonen. Querschnitte der Knospe und Scheitelansichten des Vegetationspunktes zeigen nur geringen Formwechsel; die Querschnittsform junger Stengel mit den charakteristischen Kanten ist geometrisch nächst verwandt mit der Umrissform der freien Scheitelfläche. Der Formwechsel der Phytonen verändert die gegenseitige Anordnung derselben nur in sehr einfacher Weise im Sinne einer allgemeinen Längsstreckung parallel zur Achse des Systems. Es ist deshalb erlaubt bei einer vergleichenden Betrachtung der Blattstellungen von dieser Längsstreckung zunächst abzusehen und die jungen und älteren Phytonen als lauter formgleiche Körper zu betrachten; dieses Vorgehen erleichtert die Durchführung exakter geometrischer Konstruktionen wesentlich. Wir betrachten die Phytonen als formgleiche ähnliche Körper, die nach allen Richtungen gleichmässig wachsen. Dem gleichmässigen Altersunterschied der Phytonen entspricht ein konstantes Grössenverhältnis.

Fig. 10 *a* und *b* sind Wiederholungen von Fig. 9 *a* und *b* unter Ausschaltung des Formwechsels. Die Verschiebungskurven der Blattspitzen *b*, der Blattachsen *a* und der äusseren Ecken der Blattsockel *c* sind zu geraden Linien geworden; die Abstände von *S* wachsen in gleichen Verhältnissen. So: $S_1 = S_1 : S_2 = S_2 : S_3 = \dots$. Denkt man sich die Blätter auf der Höhe der innern Blattachsel von den Blattsockeln abgeschnitten, so bleibt der Stengel zurück in der Form einer Stufenpyramide (Fig. 10 *b*). Von der Treppenstufe 5 gelangen wir in einfachem Anstieg auf die Treppenstufe 4 oder auch in doppeltem Anstieg auf die Treppenstufe 3; in dreifachem Anstieg steigen wir längs einer Pyramidenkante von Blattsockel 5 hinauf zum Blattsockel 2. Stengelquerschnitte und Scheitelfläche sind alles ähnliche Dreiecke; von jeder Ecke läuft eine Kante drei Stockwerke abwärts.

Stellt man sich vor, dass die Punkte einer Serie z. B. die Blattecken *C* in gleichmässiger Bewegung vom Scheitel *S* hinwegwandern und dabei in gleichmässiger Bewegung um die Achse rotieren, so werden die räumlichen Verschiebungskurven zu Schneckenlinien auf Kegelflächen (Fig. 11 *a*, Sch. 1928, S. 874). Vielfach, namentlich bei grosser Gliederzahl der Knospen, werden solche Schneckenlinien und Kegelflächen dem Auge unmittelbar sichtbar. Das veranlasst uns zu Konstruktionen auf Kegelflächen (Fig. 10 *c*). Wir wählen eine Stellung aus, bei welcher die Mittelkante des Blattes durch acht Stockwerke abwärts läuft bis sie vor

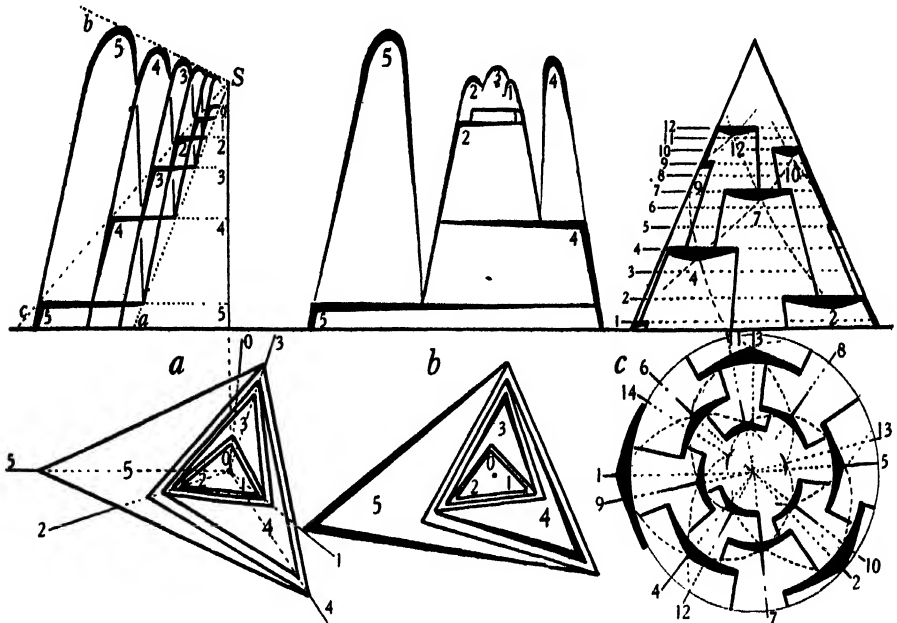


Fig. 10. Schemata für spirale Blattstellung mit einfacher Ähnlichkeit der Phytomen. (a) Aufriss, Phytomen auf einer Flanke ausgerichtet; *a*, Blattachsels; *b*, Blattspitzen; *c*, Ecken der Blattsockel. Grundriss in Form einer Stufenpyramide. (b) Grundriss und Aufriss der Stufenpyramide. (c) Konstruktion einer annähernden $3/8$ Stellung mit Divergenz 136° auf einer Kegelfläche. Kontakte 3, 5 und 8.

einem Blatt endigt. Die Reihe 1, 9, 17 ist annähernd eine Orthostiche; die Stellung nähert sich der $3/8$ Stellung mit der Divergenz 135° . Wir wählen als Divergenz 136° , also einen Zwischenwert zwischen $3/8$ und dem Grenzwinkel $137\frac{1}{2}^\circ$. Vom Zentrum aus gesehen hat jedes Blatt die Breite 60° . Daraus ergibt sich auch das Verhalten der Seitenkanten der Blätter. Die eine Seitenkante von Blatt 9 endigt drei Stockwerke tiefer vor Blatt 6, die andere Seitenkante fünf Stockwerke tiefer vor Blatt 4. Divergenz und Blattbreite bestimmen zusammen die Skulptur der Stengeloberfläche.

Wir können das Schema 10 *c* so abgeändert denken, dass die Rindenfelder nach oben in stark vorgebaute Blattsockel auslaufen; damit gelangen wir rückwärts zur Stufenpyramide ähnlich 10 *b*. Für vergleichende Betrachtungen ist es vorteilhaft sich die Kegelfläche glatt zu denken und bei gleicher Anordnung die Form der

Rindenfelder stärker zu schematisieren. Es sind punktiert einige Schrägzeilen eingetragen, welche viereckige Felder umgrenzen; in diese hinein kann man sich nach Church (1901) und van Iterson (1907) kreisförmige Blattansatzstellen denken.

(4) System der Blattstellungen

Der Spross wird vom embryonalen Vegetationspunkte aus aufgebaut als eine Kette von Phytönen, die sich in regelmässigen Altersabständen folgen und die in regelmässiger Anordnung aneinander anschliessen. Die Anordnung ist regelmässig in dem Sinne, dass jedes beliebige Glied zwischen seinen Nachbargliedern dieselbe Stellung einnimmt. Die Grundfrage des Blattstellungsproblems lautet: Warum

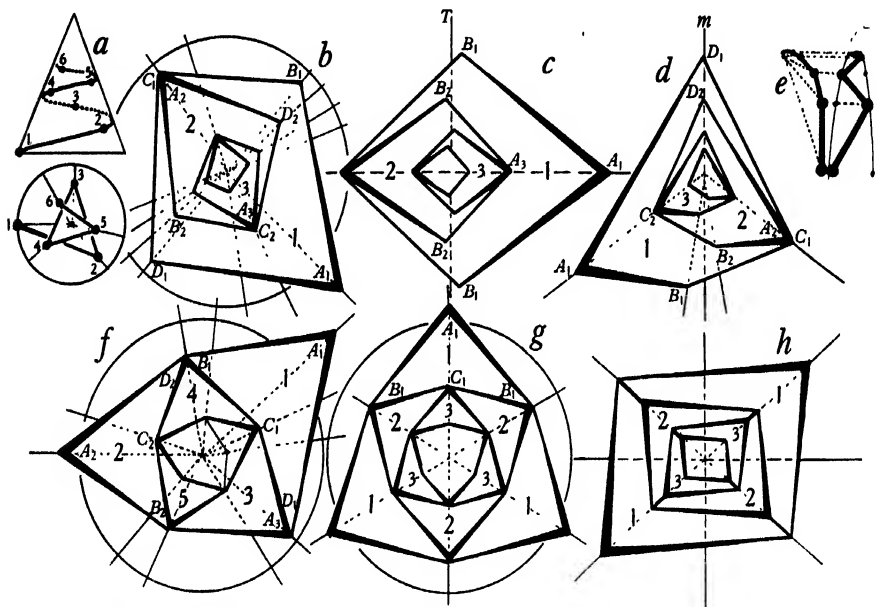


Fig. 11. System der Blattstellungen. (a) Spirale Anordnung einer Punkteschar auf einem Kreiskegel. (b) Grundriss der einfachen Spiralstellung. Divergenz 170° . (c) Zweizeilig symmetrische Stellung. (d) Zweizeilig dorsiventrals Stellung. (e) Zickzackanordnung einer Punkteschar in Seiten und Vorderansicht. (f) Zusammengesetzte Spiralstellung. Divergenz 140° . (g) Dreizählige Quirle. (h) Schiefe Quirle.

folgen sich die Phytönen in regelmässiger Anordnung und nicht einfach in dicht gedrängter zeitlicher und räumlicher Folge?

Wir stellen bewusst diese Grundfrage zurück und behandeln statt dessen die leichtere mathematisch lösbare Frage: Was für verschiedene regelmässige Anordnungen sind möglich mit Hinsicht auf die Symmetrieform der einzelnen Phytönen und die Symmetrie der Gruppen benachbarter Phytönen? Wir folgen mit dieser Fragestellung grundsätzlich dem Wege der theoretischen Kristallographie (vergl. Frey, 1925).

Fig. 11 gibt eine Übersicht über die sechs Hauptfälle (Sch. 1921, Fig. 2). Die Figuren b, c, d, f, g und h können als schematisierte Knospenquerschnitte oder als Scheitelansichten der Sprossspitze in Gestalt von Stufenpyramiden aufgefasst

werden. *b*, *c* und *d* sind einfache Stellungen mit einfacher Reihe von Phytonen; jedes Glied steht nur mit je einem älteren und einem jüngeren Gliede in Zusammenhang. Die Blätter und Blattansatzflächen sind in der Regel stengelumfassend. *f*, *g* und *h* sind zusammengesetzte Stellungen mit mehrfachen sich kreuzenden Reihen von Phytonen; jedes Glied steht mit mindestens zwei älteren und zwei jüngeren Gliedern in Zusammenhang. Die Blätter und Blattansatzflächen sind schmaler und umfassen höchstens die Hälfte des Stengelumfanges. In anderer Richtung teilen wir ein in Spiralstellungen (*b* und *f*), bei denen sich entsprechende Punkte in Spiralen oder in Schneckenlinien auf Kegelflächen folgen (Fig. 11 *a*, Sch. 1928), in symmetrische Stellungen (*c* und *g*), bei denen die einzelnen Glieder und das ganze System durch Symmetrieebenen halbiert werden (Sch. 1924, 1925) und in Zickzackstellungen, bei denen sich entsprechende Punkte in Zickzacklinien folgen (Sch. 1934, 1936); zur einfachen Zickzackstellung kommt eine Hakenkrümmung der Zickzacklinie hinzu (Fig. 11 *e*).

Einfache Spiralstellung. In Fig. 11 *b* umschliesst das Viereck A_1, B_1, C_1, D_1 ein ähnliches im Verhältnis 7 : 5 verkleinertes Viereck A_2, B_2, C_2, D_2 in beliebig gedrehter Lage. Es lässt sich zeigen (Sch. 1923, Fig. 1), dass man den Übergang von der ersten Figur zur zweiten immer auffassen kann als Verkleinerung auf ein Zentrum *Z* hin verbunden mit einer Drehung um dieses Zentrum nach einem bestimmten Divergenzwinkel. Leitet man durch fortgesetztes Verkleinern und Drehen der Figur weitere Glieder der Reihe ab, so folgen sich die Vierecke oder die zwischen denselben liegenden Blattquerschnitte in spiraliger Reihe. Dabei sind die vorausgehenden oder anodischen Ränder B_1 für Blatt 1, B_2 für Blatt 2 u. s. f. unter sich gleich und verschieden von den nachfolgenden oder kathodischen Rändern. Die Blätter sind unter sich gleichsinnig ähnlich; sie können durch Wachstum und Drehung miteinander zur Deckung gebracht werden. Der ganze Knospenquerschnitt erhält ein gedrehtes Aussehen. Man kann daher mit Goebel (1913, S. 206) von einer Scheiteltorsion sprechen; es ist aber keineswegs nötig sich mit Hirmer (1922, S. 17) asymmetrisches Wachstum zu denken. Asymmetrie der Entwicklungsfelder, die vom Scheitel abgetrennt werden, ergibt von Anfang an "kongenital" eine spirale Anordnung.

Zweizeilig dorsiventrale Stellung. In Fig. 11 *d* umschliesst wiederum das Viereck A_1, B_1, C_1, D_1 ein ähnliches im Verhältnis 7 : 5 verkleinertes Viereck A_2, B_2, C_2, D_2 in beliebiger Lage. Aber während sich die Punkte A_1 bis D_1 im Gegenzeigersinn folgen, sind die Punkte A_2 bis D_2 im Zeigersinn angeordnet. Die beiden Vierecke sind unter sich ungleichsinnig ähnlich. Es lässt sich zeigen (Sch. 1923, Fig. 2), dass man den Übergang von der ersten Figur zur zweiten immer auffassen kann als Verkleinerung auf ein Zentrum *Z* hin verbunden mit einer Umklappung um eine Mittellinie *m*. Leitet man durch fortgesetztes Verkleinern und Umklappen weitere Glieder der Reihe ab, so folgen sich die Vierecke oder die zwischen denselben liegenden Blattquerschnitte in Zickzackreihen. Dabei sind die oberen Blatt-ränder $D_1, D_2 \dots$ unter sich gleich und ebenso die unteren Ränder $B_1, B_2 \dots$. Der ganze Knospenquerschnitt weist je eine Plusseite und eine Minusseite auf (Goebel, 1913); im Zusammenhang mit dem häufig wagrechten Wachsen solcher Stengel

sprechen wir auch von Rückenseite und Bauchseite des dorsiventralen Sprosses. Ein stärkeres Wachstum der Rückenseite oder Bauchseite ist nicht notwendig mit dieser Stellung verknüpft, auch dann nicht, wenn im räumlichen Bild zur Zickzackkrümmung eine Hakenkrümmung hinzukommt (Fig. 11 e, Sch. 1934). Ungleichsinnige Asymmetrie der Entwicklungsfelder, die vom Scheitel abgetrennt werden, ergibt von Anfang an "kongenital" eine Zickzackanordnung.

Zweizeilig symmetrische Stellung. Verändern wir in Fig. 11 b oder d die beiden ersten Vierecke A_1D_1 und A_2D_2 so, dass sie sowohl für sich als auch in ihrer Verbindung symmetrische Figuren werden, so erhalten wir den Mittelfall von Fig. 11 c (Sch. 1924, Fig. 1). Die zweizeilig symmetrische Stellung vereinigt in sich Eigenschaften der beiden andern einfachen Stellungen. Es ist eine Spiralstellung mit der Divergenz 180° oder eine zweizeilige Stellung mit gleicher Ausbildung der Rückenseite und der Bauchseite. Durch Torsion wird aus der symmetrischen Stellung die Spiralstellung; durch Förderung der Plusseite gegenüber der Minusseite entsteht Dorsiventralität.

Unser Auge und unser Vorstellungsvermögen erfassen am schnellsten und am sichersten die Gesetzmässigkeiten der symmetrischen Stellung. Wir sind schon dadurch geneigt, die unsymmetrischen Stellungen aus den symmetrischen abzuleiten durch die wachstumsphysiologisch nicht gerechtfertigten Vorstellungen einer Torsion oder einseitigen Förderung. Wir werden in diesem Vorgehen bestärkt durch die Tatsache, dass sehr oft im Verlauf der Sprossmetamorphose an symmetrisch gestellte Jugendblätter spiralig oder dorsiventral gestellte Folgeblätter anschliessen. Im Sinne unserer Übersicht werden wir sagen, dass im Zusammenhang mit der reicheren Formgliederung der Folgeblätter sowohl die asymmetrischen Wachstumstendenzen des Einzelblattes als auch die Asymmetrie der Blattstellung sich stärker ausprägen.

Symmetrische Quirle. Es lässt sich zeigen (Sch. 1925, Fig. 1), dass jede mehrfache symmetrische Stellung mit exakter Symmetrie der Einzelglieder und der Nachbargruppen eine Quirlstellung sein muss. Fig. 11 b zeigt als Beispiel den dreizähligen Quirl. Die Figur ist dadurch vereinfacht, dass alle Punkte einer Schar angehören; sie verteilen sich auf zweimal drei Radian; ihre Abstände vom Zentrum nehmen ab im Verhältnis 11:7. Die durch Bezeichnung der Punkte hervorgehobene gebrochene Linie $A_1, A_2 \dots A_6$ ist eine logarithmische Spirale; das ganze Schema baut sich auf aus zweimal drei sich kreuzenden logarithmischen Spiralen. Die geometrischen Konstruktionslinien sind in diesen und vielen ähnlichen Fällen zugleich Organgrenzen; darauf stützen sich die Konstruktionen von Church (1901).

Zusammengesetzte Spiralstellungen. Das Schema (Fig. 11 f) ist in analoger Weise gezeichnet wie Fig. 11 g. Die Punkteschar verteilt sich auf Radian mit einer Divergenz von 140° ; sie liegt also zwischen einer $2/5$ Stellung mit der Divergenz 144° und der Limitdivergenz $137\frac{1}{2}^\circ$. Die Abstände der Punkte A vom Zentrum nehmen ab im Verhältnis 17:14. Je drei Glieder der Grundspirale umschliessen das Knospeninnere so, dass an zwei Stellen Eckenkontakt eintritt; je fünf Glieder der Grundspirale umschliessen das Knospeninnere vollständig durch

Übereinanderschieben der Flanken. Ein Umgang von fünf Gliedern entspricht zwei dreizähligen Quirlen in Fig. 11 g.

Wir haben in Fig. 11 f wie in Fig. 9 und 10 den allgemeinen Fall der zusammengesetzten Spiralstellung vor uns mit asymmetrischer Gestalt der Einzelglieder. Die anodischen Ränder B_1, B_2, \dots sind schmaler aber dicker als die kathodischen Ränder D_1, D_2, \dots . Ebenso ist die Kontaktzeile 1, 4, 7 steiler als die Kontaktzeile 1, 3, 5, 7. Nun gilt für die asymmetrischen Stellungen, dass sie mit wenig Ausnahmen sich stark an verwandte symmetrische Stellungen annähern. Die Glieder 4 und 3 schliessen in ähnlicher Weise an 1 an, wie im dreizähligen Quirl zwei Glieder 2 an ein Glied 1. Aus grosser Annäherung an die symmetrische Ordnung lässt sich die Bevorzugung von Winkeln nahe der Limitdivergenz verständlich machen (van Iterson, 1907; Hirmer, 1931-4; Snow, 1931-5; Sch. 1936, Fig. 14).

Schiefe Quirle (Fig. 11 h, Sch. 1936). Schiefe, zweizählige Quirle kommen vor als seltene Blattstellung in der Familie der Crassulaceen; sie bilden aber eine notwendige Ergänzung unseres Systems. Je zwei Blätter sind gleich hoch inseriert, gleich gross und im gleichen Sinne asymmetrisch. Die Blätter des folgenden Quirls sind im entgegengesetzten Sinne asymmetrisch. Statt logarithmischer Spiralen treten Zickzacklinien als leitende Konstruktionslinien hervor.

Wir finden als Resultat eines Überblicks über die Gesamtheit der Blattstellungen: Die in der Natur verwirklichten Stellungen lassen sich einordnen in ein logisch, geometrisch begründetes System. Sie sind Lösungen der Aufgabe, aus gleichsinnig oder ungleichsinnig asymmetrischen oder aus symmetrischen Teilkörpern Anordnungen zu bilden, die sich unverändert und unbegrenzt fortsetzen lassen. Dabei sind diejenigen Stellungen in der Häufigkeit bevorzugt, in denen strenge Symmetrie oder doch eine grosse Annäherung an strenge Symmetrie verwirklicht werden kann.

Entwicklungsgeschichtlich ist die Blattstellung zurückzuführen auf die Zerlegung der Scheitelfläche in Entwicklungsfelder der Blattanlagen. Diese Entwicklungsfelder zeigen von Anfang an in Form und gegenseitiger Stellung die charakteristischen Eigenschaften der Art und des Blattstellungsgesetzes; beim Auswachsen der Phytone prägen sich diese Symmetrieeigenschaften in verschärfter Weise aus. In Ergänzung dessen, was weiter oben über die innere Struktur des Vegetationskegels gesagt wurde, können wir uns folgendes Bild machen (Sch. 1925, S. 413). Jedes Entwicklungsfeld eines werdenden Blattes hat innerhalb der Fläche geordnete Protoplasmamoleküle. Diese Ordnung bestimmt die Anisotropie des Wachstums nach verschiedenen Radien vom Blattzentrum aus und damit die Symmetrieform des werdenden Blattes. Die Ordnungen der einzelnen Entwicklungsfelder stehen zueinander in bestimmten Beziehungen etwa wie die Ordnungen der Raumgitter in verwachsenen Kristallzwillingen. Ohne heute eine bestimmte Ausgestaltung dieser Ansichten zu entwickeln, möchte ich nachdrücklich auf die allgemeine Übereinstimmung zwischen Blattstellungssystem und System der Kristalle hinweisen.

III. VERZWEIGUNG

(1) *Periodizität der Sprossverzweigung*

Bei der Blattbildung steht der embryonale Vegetationspunkt als Einheit der Kette der Phytomen gegenüber. Die Sprossverzweigung der Angiospermen geschieht in Abhängigkeit von der Blattbildung. Das Urmeristem der Achselsprosse entsteht in der vegetativen Region regressiv; die Achselvegetationspunkte sind Neubildungen. Aus der Art ihrer Entstehung folgt, dass sie zunächst geringere Masse haben als der Vegetationspunkt des Muttersprosses (vergl. in Fig. 1 a die Achselsprosse A_3 mit VP). Sie sind erst nach einer gewissen Wachstumsdauer zur Blattbildung befähigt. Die Achselsprosse wiederholen die Wachstumsvorgänge der Muttersprosse mit einer gewissen Verspätung.

Die vollständigste Ausbildung vegetativer Sprosssysteme beobachten wir bei den Stockausschlägen (Sch. 1918, 1929). Hier kommt es vor, dass alle Seitenachsen ebenso kräftig oder fast ebenso kräftig auswachsen wie ihre Mutterachsen. Es können durch die Reservestoffe des Baumstumpfes und die reiche Versorgung von den Wurzeln her für viele Knospen längere Zeit hindurch optimale Wachstumsbedingungen aufrecht erhalten bleiben, bei welchen auch die hemmenden Wirkungen der Knospen aufeinander nicht zur Geltung kommen. Die Seitenzweige setzen mit einer charakteristischen Verspätung ein; diese beträgt beispielsweise fünf bis sechs Plastochron bei *Quercus robur* und *Juglans nigra*, zwei Plastochron bei *Fraxinus excelsior*, *Fagus silvatica* und *Acer pseudoplatanus*.

Häufig beginnen die Meristeme in den Blattachsen mit der Blattbildung schon bevor sie die Grösse des Muttervegetationspunktes erreicht haben. Ihre ersten Blattanlagen sind dann auch kleiner als die Blattanlagen am Mutterspross. Aus diesen kleinen Anlagen gehen einfache Jugendblätter hervor, an welche bald die normalen Folgeblätter anschliessen. Einfache Jugendblätter müssen sich auch unter optimalen Bedingungen einstellen, solange der Vegetationspunkt im Erstarken begriffen ist; sie sind keine Hemmungsbildungen.

Unsere Fig. 12 a zeigt den Typus eines solchen Verzweigungssystems mit einer Verspätung der Seitenachsen von zwei Plastochron. An der Hauptachse ist die Verzweigung bis zum Knoten 9 fortgeschritten; die unmittelbar der Hauptachse entspringenden Seitenachsen erster Ordnung I bis IX sind verzweigt bis zu Knoten 7; die Seitenachsen zweiter Ordnung sind verzweigt bis zu Knoten 5. Jede Seitenachse mit ihren Verzweigungen ist gleich einem Endabschnitt der Hauptachse. Das ganze System kann wachsen ohne seinen Charakter zu verändern.

Diese einfachste und vollständigste Ausbildung eines Verzweigungssystems ist ein Grenzfall, der in der Natur nur unter optimalen Bedingungen annähernd verwirklicht wird. Im Zusammenhang mit beschränkter Ernährung, durch das Eingreifen von Wuchsstoffhemmungen, durch den jahreszeitlichen Wechsel der äusseren Bedingungen kommen eingeschränkte Entwicklungen der Sprosssysteme zustande. Darüber sei nach Untersuchungen an *Acer pseudoplatanus* (Sch. 1929) Folgendes angeführt.

Die Anlage der beiden ersten besonders grossen und reich gegliederten Blattpaare der Langtriebe erfolgt im Juli; beim Austreiben im nächsten Frühjahr folgen sich diese beiden Paare mit sehr geringem Entwicklungsabstand. Weitere Blattpaare folgen in regelmässigen Entwicklungsabständen; ihre Entfaltung kann sich bis in den August ausdehnen. Kurztriebe lassen nur noch ein schwächeres drittes Blattpaar folgen. Das Aufhören der Laubblattentfaltung bedeutet keineswegs Wachstumsstillstand am Vegetationspunkt; dort werden nun anstelle der Laubblattanlagen Schuppenanlagen abgegliedert. Diese unterscheiden sich gegenüber

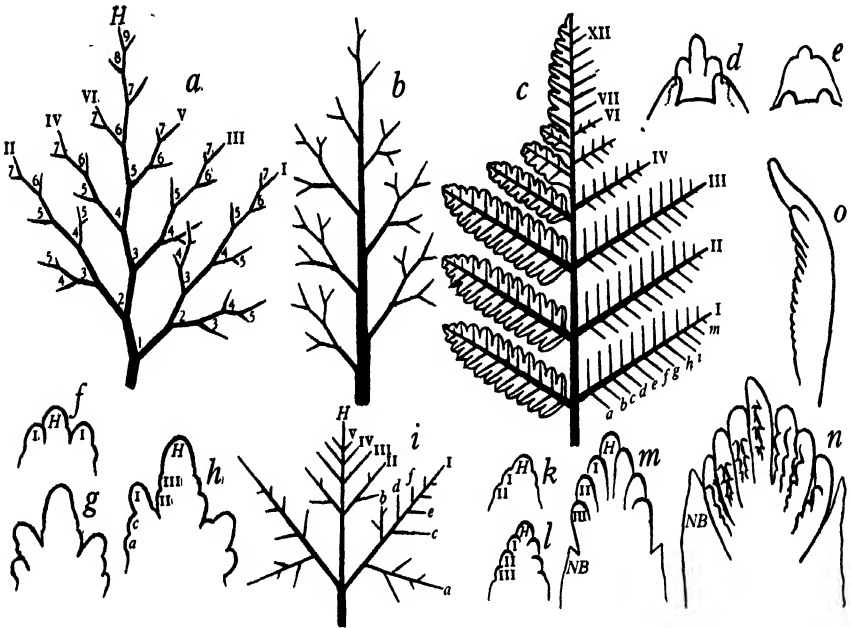


Fig. 12. Blattverzweigung. (a) Bäumchenform. H, Hauptachse; I bis IX Glieder erster Ordnung. 1 bis 9 Knoten, von der gemeinsamen Basis aus nummeriert. (b) Fiedertypus durch Begrenzung der Seitenglieder. (c) Fiedertypus mit Metamorphose längs der Hauptachse und mit vorgreifender Ausbildung der Seitenachsen. (d) Laubblattanlagen und (e) Schuppenanlagen von *Acer pseudo-platanus*. (f) bis (i) Blattentwicklung von *Aconitum napellus*; bei (i) Verzweigungsschema einer älteren Blattanlage. (k) bis (o) Blattentwicklung von *Rosa* species NB, Nebenblätter, bei (o) einzelnes Fiederblatt.

den Laubblattanlagen von den ersten Entwicklungsstadien an. Bei den Laubblattanlagen überwiegt früh der gegliederte Spreitenteil (Fig. 12 d). Bei den Schuppenanlagen überwiegt die ungegliederte Blattbasis (Fig. 12 e). Nur bei den äusseren Schuppen spielen Verkümmern und Absterben der Spreiten eine direkte Rolle; die inneren Schuppen haben ihren eigenen Entwicklungsgang mit kurzer Wachstumsdauer. Ebenso bleiben in der Knospenspur die Internodien kurz, und die Achselsprosse erscheinen spät oder fehlen.

Gegenüber dem vollständigen Sprosssysteme der Stockausschläge bleiben am Langtrieb die Achselsprosse mehr und mehr in der Entwicklung zurück und gehen frühzeitig zur Knospenbildung über. Beseitigt man noch während des Frühjahrswachstums die fortwachsende Endknospe und hebt man damit die Hemmungen für

die Seitenknospen auf, so kann man in diesen die Bildung von Knospenschuppen wiederum durch die Bildung von Laubblättern ersetzen. Aus schon vorhandenen Anlagen entstehen bei der Umschaltung charakteristische Zwischenformen.

Den wachstumsphysiologischen Unterschied von Hauptsprossen und Seitensprossen zeigen auch einjährige Pflanzen wie *Lathyrus ochrus* (Sch. 1927). Zunächst hat der Hauptspross einen Vorsprung von sechs Plastochron wegen der verspäteten Anlage der Seitensprosse. Ausserdem arbeitet der Vegetationspunkt des Hauptsprosses rascher; er hat ein kürzeres Plastochron als die Vegetationspunkte der Seitensprosse und lässt diese mehr und mehr hinter sich zurückbleiben. Ist beispielsweise der Hauptspross fortgeschritten bis zur Anlage von Blatt 50, so sollten alle Seitensprossen von der gemeinsamen Basis des Kotyledonarknotens aus gezählt bis zur Anlage der Blätter 44 fortgeschritten sein. Es gelangte aber Seitenspross 1 nur bis zur Anlage 10, Spross 10 bis zur Anlage 21, Spross 20 bis zur Anlage 30, Spross 30 bis zur Anlage 37. Trotz starker Hemmung in bezug auf Anlage und Auswachsen der Phytomenen hat auch in den Seitensprossen wie im Hauptspross die Metamorphose der Blattformen und der Übergang zur Blühreife stattgefunden; es kommt aber in den Achselknospen nicht zur Entfaltung der Blüten. Nach frühzeitigem Entfernen der Sprossspitze übernehmen die obersten Achselprosse wachstumsphysiologisch die Rolle des Hauptsprosses.

(2) Periodizität der Blattverzweigung

Auch bei der Gliederung der Blattspreiten spielt die periodische Wiederholung gleicher oder ähnlicher Teile eine grosse Rolle. Da alle Verzweigungen in einer Fläche liegen, ist die zeichnerische Darstellung viel leichter als bei der Sprossgliederung; andererseits sind die Verhältnisse dadurch kompliziert, dass im Laufe der Ausbildung des Blattes der Charakter der Verzweigung sich verändert. Unter der grossen Mannigfaltigkeit der Blatttypen lassen sich immerhin einige einfache Typen herausgreifen und in ihrer Gesetzmässigkeit darstellen. Ich nenne das gefiederte Blatt, das fingerförmig zusammengesetzte Blatt, das parallelnervige Blatt. Durch die Vorstellung einer allmählichen Umformung eines Typus in einen andern lässt sich die ganze Mannigfaltigkeit ordnen (Sch. 1919). Heidenhain (1932) hat in ausgezeichneter Weise die weite Verbreitung gabeliger Verzweigung bei den Blättern nachgewiesen. In ähnlicher Art soll im Folgenden auf die besondern Merkmale des gefiederten Typus kurz hingewiesen werden (vergl. Troll, 1935).

Wir gehen aus von der Bäumchenform, Fig. 12 a, wie sie bei Sprossen und auch bei primitiven Farnwedeln vorkommt. Alle Spitzen sind in Fortbildung begriffen. In regelmässigen Zeitabständen, in Plastochronen der Blattverzweigung, erleidet die Spitze eine Ungleichteilung. Der stärkere Gabelast oder Hauptzweig stellt sich annähernd in Verlängerung der Mutterachse; der schwächere Gabelast oder Seitenzweig wird von der Richtung der Mutterachse stärker abgelenkt, gleichsam vom Hauptzweig beiseite gedrängt. Der Hauptvegetationspunkt ist rasch regeneriert und zu neuer Verzweigung befähigt; der schwächere seitliche Vegetationspunkt muss während einer längeren Periode z. B. während drei Plastochron heranwachsen und erstarken, um sich ebenfalls verzweigen zu können. Vom Zeitpunkt

seiner ersten Verzweigung ab ist der Seitenzweig der Mutterachse gleichwertig geworden. Wenn ein solches Verzweigungssystem nach einer gewissen Entwicklungsdauer mit allen seinen Spitzen in den Dauerzustand übergeht, stellt es ein bäumchenförmig zusammengesetztes Blatt dar; dies ist ein schematisierter einfacher Grenzfall der Blattbildung.

Zum Typus Fig. 12 *b* führen uns folgende Abänderungen. Der Hauptvegetationspunkt des Blattes ist ebenfalls befähigt längere Zeit hindurch periodisch schwächere Seitenvegetationspunkte abzugliedern. Diese Seitenvegetationspunkte erster Ordnung bleiben aber vom Hauptvegetationspunkte verschieden; sie haben von vornherein eine enger begrenzte Entwicklungsfähigkeit und stellen nach Bildung einer kleineren Zahl von Seitenvegetationspunkten zweiter Ordnung die weitere Verzweigung ein. Noch enger begrenzt ist die Entwicklung der Seitenvegetationspunkte dritter Ordnung. Der starke Hauptvegetationspunkt hat in der Verzweigung einen grossen Vorsprung vor den Seitenvegetationspunkten erster Ordnung; diese haben nur noch einen kleinen oder keinen Vorsprung vor den Seitenvegetationspunkten zweiter und dritter Ordnung. Die Hauptachse ist seitlich verzweigt, gefiedert; die schwächeren Seitenachsen sind gabelig verzweigt. Wenn schliesslich die Hauptachse ihre Fortbildung einstellt, so bleiben auch die jüngsten Seitenachsen erster Ordnung auf einer früheren Verzweigungsstufe stehen; das gefiederte Blatt endigt in eine Bäumchenform.

Zum verbreiteten Typus des Fiederblattes, Fig. 12 *c*, führen weitere Abänderungen. Wir bezeichnen dieselben mit Nägeli (1846) als Metamorphosen innerhalb des Blattes, als Folgen einer Umwandlung der Tätigkeit des Blattvegetationspunktes während der individuellen Blattbildung. Der Hauptvegetationspunkt des Blattes bildet zuerst einige Paare kräftiger Seitenvegetationspunkte I bis III; diese wachsen zu kräftigen Fiederästen aus und zwischen ihnen bilden sich lange Internodien einer kräftigen Mittelrippe ohne Spreitensaum. Später bildet der Hauptvegetationspunkt Seitenvegetationspunkte erster Ordnung IV bis VI mit abnehmender Verzweigungskraft und lässt zwischen denselben kürzere, schwächere Internodien auswachsen. Schliesslich folgen dicht hintereinander schwache Seitenzweige erster Ordnung VII bis XIII mit Spreitenbildung, und auch die Hauptachse selbst trägt eine Endspreite. Die Seitenzweige erster Ordnung sind Wiederholungen der Mutterachse, aber unvollständige Wiederholungen mit Auslassung der Anfänge der Metamorphose; sie entsprechen bloss dem Endteil der Mutterachse. Die Zweige I bis III beginnen mit dichtgestellten kurzen und mit Spreiten versehenen Zweigen zweiter Ordnung *a* bis *h* und endigen nach einigen schwächeren Zweigen *i* bis *m* mit Endspreiten. Die Zweige IV bis VI bilden den Übergang von den verzweigten Gliedern I bis III zu den unverzweigten Gliedern VII bis XIII. Zwei Erscheinungen verleihen also dem Typus 12 *c* seine Eigenart. Längs einer Achse finden wir Metamorphose sowohl in bezug auf Grösse, Wertigkeit und Abstand der Seitenglieder als auch in bezug auf Ausbildung von Rippe und Spreitensaum. Die Teilungen des Vegetationspunktes in Muttervegetationspunkt und Seitenvegetationspunkt sind Ungleichteilungen in dem doppelten Sinne, dass der Muttervegetationspunkt eine grössere Entwicklungsfähigkeit behält und dass der

Seitenvegetationspunkt mit einer Stufe der Metamorphose einsetzt, welche vom Muttervegetationspunkt erst später erreicht wird.

Die Übergangsregion der Zweige IV bis VI der Fig. 12 c, die bei Farnwedeln meist sehr reich entwickelt ist, kann ausfallen; an die starken weiterverzweigten Glieder I bis III können unmittelbar die schwachen nicht mehr verzweigten Glieder VII bis XIII anschliessen. Dieser Typus des unpaarig gefiederten Blattes ist bei Dikotylen häufig (Fig. 12 n).

Die formal-morphologischen Betrachtungen, die wir eben durchführten, müssen durch entwicklungsgeschichtliche Beobachtungen ergänzt werden. Dafür ist heute noch zurückzugreifen auf die Arbeiten von C. Nägeli 1845 bis 1855 (Sch. 1933) und A. Eichler 1861. Um einige allgemeine Gesichtspunkte hervorzuheben vergleichen wir die Blattentwicklung von *Aconitum napellus*, Fig. 12 f bis i, und einer *Rosa species*, Fig. 12 k bis o.

Das Blatt von *Aconitum* gehört einem weitverbreiteten Typus an, der zwischen Bäumchenform, mehrfacher Fiederung, mehrfach dreizähliger Verzweigung und zwischen dem fünfstrahlig fingerförmigen Blatt in der Mitte steht; es ist handförmig gelappt, gespalten bis geteilt. Wir können nach Symmetrie und Verzweigung eine Hauptachse und Glieder erster, zweiter und höherer Ordnung unterscheiden; nach ihrer Ausbildung bilden alle Glieder eine geschlossene Übergangsreihe vom kleinen Randzahn bis zum grossen selbständigen Spreitenteil. Das ist darauf zurückzuführen, dass sowohl der ursprüngliche Hauptvegetationspunkt des Blattes als auch alle später entstehenden Seitenvegetationspunkte sich längere Zeit hindurch fortbilden und weiter verzweigen. Der Hauptvegetationspunkt erfährt jeweils eine Dreigliederung, wobei immerhin der Mittelteil als Fortsetzung der Hauptachse einen Vorsprung behält. Die andern Vegetationspunkte zeigen alternierend seitliche bis gabelige Verzweigung, wobei namentlich die ersten Zweige auf der Aussenseite stark ausgebildet werden. Die Begrenzung der Entwicklung erfolgt an allen Gliedern ungefähr gleichzeitig; daher rührt die Annäherung an die Bäumchenform. Genauer betrachtet gehen zuerst der Hauptvegetationspunkt und zuletzt die basalen Seitenglieder in den Dauerzustand über.

Einen ganz andern Typus mit scharfer Unterscheidung verschiedener Gliederungen zeigt das Blatt von *Rosa*. Es ist unpaarig gefiedert; der Endteil der Hauptachse ist den seitlichen Fiedernblätter gleichwertig und darf deshalb mit zu den Gliedern erster Ordnung gerechnet werden. Die sieben Fiederblättchen tragen als Glieder zweiter Ordnung sägeförmige Randzähne. Übergänge zwischen Fiederblättchen und Randzähnen fehlen. Am jungen Blatt entstehen rasch hintereinander die Blattfiedern (Fig. 12 k, l). Nach längerem Wachstum beginnt an allen Fiedern gleichzeitig die Bildung der Randzähne (Fig. 12 n). Hier gilt die Aussage von Eichler (1861, S. 10). "In den allermeisten Fällen spricht sich in der Zeit der Entstehung der Spreitenglieder verschiedener Ordnung eine bestimmte Periodizität aus und zwar in der Art, dass kein Glied einer höheren Ordnung früher angelegt wird ehe alle Glieder der nächst niederen Ordnung fertig gebildet sind."

Wir schliessen damit unsern Überblick über periodische Formbildung der Pflanzen. Er zeigt eine Mannigfaltigkeit von weiteren Forschungsmöglichkeiten.

Die Weiterbildung der Morphologie beruht darauf, dass wir immer besser lernen, die Formen als Ausdruck von Wachstumsordnungen zu erfassen. In diesen Wachstumsordnungen spricht sich neben entwicklungsphysiologischen Zusammenhängen vor allem das erbliche Wesen der Pflanzenarten aus.

IV. ZUSAMMENFASSUNG

I. *Ontogenie*. Aus der Laubknospe heraus werden periodisch Blätter entfaltet. Dieser Vorgang ist in seinem zeitlichen Verlauf ein genaues Abbild für die periodische Entstehung neuer Blattanlagen aus der ungegliederten Masse des Vegetationspunktes. Der Vegetationspunkt macht bei stetigem Wachstum einen periodischen Formwechsel durch. Askenasy hat schon 1880 auf diese Periodizität des Knospenswachstums eine Methode der Wachstumsmessung gegründet; seine Methode ist von grundlegender Bedeutung für den Ausbau einer exakten Entwicklungsgeschichte.

Nach den Richtungen der Zellteilung und des Wachstums lassen sich drei Hauptarten der Meristeme unterscheiden. Die massigen Meristeme im Innern der Vegetationspunkte und in den Sporangien teilen sich und wachsen gleichmässig nach den drei Richtungen des Raumes. Die Rippenmeristeme der Wurzeln, Stengel, Blattstiele und Blattrippen teilen sich und wachsen vorwiegend oder ausschliesslich in der Richtung der Längsachse des Organs. Die Plattenmeristeme der Blattspreiten teilen sich und wachsen fast ausschliesslich parallel zur Blattfläche. Im Vegetationspunkt wachsen die oberflächlichen Schichten der Tunica nach der Art eines Plattenmeristems; der Kern des Vegetationspunktes wächst als massiges Meristem; das junge Stengelmark wächst als Rippenmeristem. Bei der Blattbildung falten sich die Oberflächenschichten; dadurch werden das Flächenwachstum der Oberfläche und das Dickenwachstum des Innern aneinander angepasst.

II. *Blattstellung*. Ein Blatt und das tragende Stengelglied bilden miteinander eine Wachstumseinheit, ein Phyton. Der Aufbau des Sprosses aus Phytönen ist phylogenetisch betrachtet das Endglied einer langen Entwicklungsreihe. Diese fängt an mit einem mikrophyllen Spross, einer Achse die kleine haarförmige oder schuppenförmige Anhängsel trägt, oder auch mit einem gabelig verzweigten Thallus; an der Spitze der Achse oder an den Spitzen der Thalluszweige findet sich je eine kleine Teilungs- und Wachstumszone. Der phylogenetische Fortschritt beruht auf einer Ausdehnung dieser Teilungs- und Wachstumszone. Dadurch entsteht ein gemeinsames Wachstum und eine gegenseitige Beeinflussung ursprünglich selbständiger Teile. Durch den Einfluss des Blattes auf den tragenden Stengelteil entsteht die neue entwicklungsphysiologische Einheit des Phyton.

Das Problem der Blattstellung wird behandelt als mathematisches Problem der Anordnung formgleicher, wachsender Teilkörper, der Phytönen. Dabei wird der Zusammenhang zwischen Symmetrie der Anordnung und Symmetrie der Ausbildung hervorgehoben. Es entsteht ein System der Blattstellungen, das mit dem System der Symmetrieklassen der Kristalle nahe verwandt ist.

III. *Verzweigung*. Die Vegetationspunkte der Seitensprosse entstehen als Neubildungen in bestimmtem räumlichem und zeitlichem Abstand vom Vegetations-

punkt der Mutterachse. Daraus ergeben sich einfache Regeln für die Verzweigung die besonders bei kräftig wachsenden Stockausschlägen klar verwirklicht werden. Ähnliche Regeln gelten für Blätter mit einfachem "bäumchenförmigem" Verzweigungsschema.

Die Metamorphose der Blätter längs einer Sprossachse ist zurückzuführen auf eine innere Metamorphose des Sprossvegetationspunktes. Reichgegliederte Fiederblätter zeigen eine Metamorphose der Blättchen längs der Mittelrippe; diese ist zurückzuführen auf eine innere Metamorphose des Blattvegetationspunktes.

IV. Das allgemeine Ziel, das in den referierten Arbeiten verfolgt wird, ist eine Weiterbildung der formalen Morphologie durch eine messende Entwicklungsgeschichte. Jede Form muss erfasst und beschrieben werden als ein Ergebnis des Wachstums und als Ausdruck einer Wachstumsordnung.

V. SUMMARY

(1) *Ontogeny*. Leaves are periodically developed from leaf-buds. This process, as regards its time relations, is a precise copy of the periodic development of new leaf-rudiments from the undifferentiated growing point. The latter, growing constantly, undergoes a periodic form-change. As early as 1880 Askenasy based a method of growth-measurement on this periodicity in the growth of buds; his method is of fundamental importance to embryology.

Three main types of meristem can be distinguished by the directions of cell-division and growth. The massive meristems inside the growing point and the sporangia divide and grow uniformly in the three directions of space. The rib-like meristems of roots, stems, petioles and veins divide and grow mainly or exclusively in the direction of the longitudinal axis of the organ. The lamellar meristems of the leaf-blade divide and grow almost exclusively parallel to the surface of the leaf. In the growing point the superficial layers of the tunica grow as a lamellar meristem; the centre of the growing point grows as a massive meristem; the young pith of the stem grows as a rib-like meristem. In the development of the leaf the superficial layers fold up. The growth in area of the surface and the growth in thickness of the inside are thus adapted to one another.

(2) *Phyllotaxis*. A leaf and the part of the stem which bears it together form a growth unit, a so-called phyton. From the phylogenetic point of view the formation of the shoot out of phytons is the final stage in a long process of development. This process starts with a microphyllous shoot, an axis bearing small hair-like or scale-like appendages, or with a bifurcated thallus. At the tip of the axis or of the branches of the thallus there is a small zone of division or growth. The phylogenetic progress consists in an extension of these zones of division or growth. Thus there comes about a unified growth and a mutual influence between originally independent parts. The new developmental unit, the phyton, owes its origin to the influence of the leaf on the part of the stem bearing it.

The problem of phyllotaxis has been dealt with as a mathematical problem of the arrangement of growing parts of identical form, the phytons. The connexion between symmetry of arrangement and symmetry of form is thereby emphasized. A system of phyllotaxes thus arises which is nearly related to the system of symmetry classes in crystals.

(3) *Branching*. The growing points of the lateral branches originate as new structures having a definite spatial and temporal relation to the growing point of the mother axis. Simple rules of branching can be deduced from this, which are particularly well shown by powerfully growing adventitious shoots from tree stumps. Similar rules hold for leaves with a simple tree-like manner of branching.

The metamorphosis of leaves along the axis of a shoot can be traced back to an internal metamorphosis of the growing point of the shoot. Pinnate leaves show a metamorphosis of the leaflets along the midrib. This can be traced back to an internal metamorphosis of the growing point of the leaf.

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ENZYME VARIATION IN MICRO-ORGANISMS

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I. INTRODUCTION. VARIATION IN MICRO-ORGANISMS

AMONGST the higher organisms, variation is usually considered as differences amongst individuals. When dealing with micro-organisms, where observations are almost always recorded from huge populations, such individual variations cannot be appreciated and the term variation must here mean a general change in the average characteristics of the cells under investigation.

Biological variation in all types of organisms may be conditioned by alterations in the environment. In many instances, the change is in the nature of an adaptation to the altered conditions, the variants being biologically more suited to the changed environment than the original organisms. Adaptation of this nature is a characteristic property of living organisms. D'Herelle, in attempting to prove the living nature of bacteriophage, bases his discussions chiefly on this fundamental tendency of living matter to become adapted to an altered environment.

It is an axiom that bacteria have no physiology, only a biochemistry. Of especial interest then in the study of variation in micro-organisms is the variation in their enzymic make-up. In many instances, micro-organisms produce enzymes *de novo*, or in increased amounts, in the presence of the specific substrates of the enzymes and these instances may be looked upon as examples of chemical adaptation. The terms mutation, modification, acclimatization, training and adaptation have all been used to describe examples of this nature. Karström (1930) classifies enzymes as "adaptive" or "constitutive", the former being formed only in the presence of the enzyme substrate. Since the mechanism of the production of the enzymes in the

presence of the substrate may be of more than one type and since moreover enzyme production may be conditioned by substances unrelated to the substrate, it might be of interest to consider some of the possible mechanisms whereby enzymes might arise in cultures previously not possessing them.

II. BIOLOGICAL TYPES OF ENZYME PRODUCTION

In those instances where enzyme production is associated with the presence of its substrate, there are two theoretical possibilities regarding the mechanism by which this production may be brought about.

In a paper published in 1932, these possibilities were discussed by the writer as follows: "The influence of the substrate on the production of organisms possessing the enzyme may be pictured as either a natural selection or a chemical adaptation. In the former case, one must suppose that there exists in all cultures a small but definite number of cells possessing the enzyme. Since at some period each of the organisms used has been grown from a single cell colony, one must imagine a biochemical variation or mutation of a definite though low frequency, in order to account for the existence of cells containing the enzyme. Enzymes arising by some such mutation become, according to this hypothesis of natural selection, of biological value to the organism. Those members possessing them are therefore at an advantage and tend to multiply at the expense of the others. A strain is thus formed in which the majority of the members possess the enzyme in question. The second suggestion is that of chemical adaptation. On this view, an adaptive enzyme arises as a response to its chemical environment. It would then partake of the nature of an acquired character in higher organisms. With the removal of the stimulus, the character is lost by the descendants of the organism. They still, of course, retain the power to develop that character in conditions in which the parent organism developed it.

"In this way, one would distinguish 'training' and 'adaptation' by considering the former to be an inheritable variation, whilst the latter is a specific response to a change in the environment and is hence of the nature of a non-inheritable acquired character."

The enzyme being studied at that time was the hydrogenlyase of *Bact. coli*, the enzyme responsible for the liberation of hydrogen from glucose or formic acid. Chiefly from theoretical considerations, it was then decided that the production of this enzyme was not due to natural selection, i.e. it was not produced by "training" but by "adaptation". Soon afterwards, this view was confirmed experimentally by Stephenson & Stickland (1933), who showed that formic hydrogenlyase could be produced by *Bact. coli* in the absence of cell division, i.e. in conditions in which selection could not possibly operate.

More recently, Stephenson & Yudkin (1936) were able to show in another instance that enzyme production can occur as a direct interaction of cell and substrate. It is well known that certain strains of yeast exist, which, although not originally capable of fermenting galactose, are able to do so when grown for a short time in the presence of this sugar. By following the production of the enzyme in

yeast suspended in a solution of galactose, galactozymase formation was obtained in the complete absence of growth, as determined by total and viable counts.

Here then we have two examples of enzymes being produced in living, non-dividing cells as a direct response of those cells to a specific chemical environment. On the other hand, examples of the second mode of enzyme formation in the presence of the substrate—namely, that of natural selection or “training”—are not lacking. Since the report of Neisser (1906) and Massini (1907), many instances have been recorded of the development among coliform organisms of lactose fermenting colonies from strains originally unable to ferment this sugar. Massini himself considered the appearance of the fermenting bacteria as a mutation and called his strain *Bact. coli mutabile*.

There are two possible modes of origin of these mutating cells. First, the mutation may occur continuously, irrespective of the medium; that is, as suggested above, there would always be present in any given culture a few cells possessing the new enzyme. On the other hand, the presence of the substrate, in this case lactose, might act as a specific stimulus for the production of the mutation, as well as later supplying a factor for the selecting out of the mutant cells. This second possibility is really a combination of “adaptation” and “training”.

This question has been definitely answered by Lewis (1934). Working with several *mutabile* strains of colon bacteria, he was able to show that the variation occurred independently of the presence of the sugar. For example, one of his strains, which produced secondary lactose-fermenting colonies on lactose broth agar, was unable to grow on a synthetic lactose agar medium when sown in the ordinary way. However, by plating on to such a medium from very low dilutions, a few colonies did develop; and by comparative counts on this synthetic medium and on a broth medium, Lewis estimated that about one cell in 100,000 in any given culture was capable of utilizing lactose on an inorganic medium. That is, even in the absence of lactose, cells are always being produced, though only in small numbers, which show this “lactose mutation”. Similar results were obtained with other bacteria, variable to lactose, sucrose, raffinose or rhamnose. In these other strains, although growth did occur on the synthetic medium, the normal colonies produced were very small but occasional larger variant colonies appeared. The presence of the compound to which variation occurs did not increase the proportion of variant colonies and Lewis concludes that “the sugars act as selective agents but do not exert a specific exciting stimulus to variation”.

The “training” of an organism to perform new reactions, or the enhancement of reactions normally but slowly performed, is often possible by serial subculture into media containing the new substrate. Organisms may also be “trained” to grow in media previously insufficient to support growth and, as Knight and Fildes (see Knight, 1936) have pointed out, this may be regarded as due to the development in the organisms of the requisite synthetic enzymes. These cases of “training” or enhancement may be brought about by either of the mechanisms just discussed—adaptation or selection. In many instances, it appears that the mechanism of enzyme production is that of selection. For example, it has often been recorded that

attempts to train any given strain of organism succeed in only a few of several simultaneous experiments and that the attempts are more likely to be successful if large inocula are used. Only with such large inocula would there be a fair chance of carrying over a few of the mutant cells into the fresh medium. Thus, Lewis found that one of his strains which fermented lactose only after a long lag period, could be induced to ferment it more rapidly if subcultured with large inocula which by calculation were found to be large enough to contain some of the mutant cells.

In these instances then, training consists of selecting out one type of cell from what might almost be considered a mixed culture. Each subculture into the appropriate medium increases the proportion of the mutant cells since these are at a biological advantage in that they have at their disposal an additional source of energy or of material for the building up of their protoplasm.

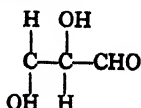
The results of Penfold (1911) with a strain of *Bact. paratyphosum* are of interest in this respect. Bacteria sown from an ordinary peptone medium into dulcitate-peptone began to ferment the dulcitate after about 10 days. Subculturing from this into fresh dulcitate-peptone resulted in fermentation appearing after 1 or 2 days. Plating a peptone culture on to a neutral red dulcitate plate gave a small number of red (i.e. dulcitate-fermenting) colonies. A much larger number was obtained from a dulcitate-peptone culture.

Interesting results were obtained by counting experiments in a dulcitate medium. After a few days, during which the increase in the viable count was normal, a sudden large increase in the cell number took place. In the first part of the experiment, plating showed the usual small proportion of red colonies. At the point where the count suddenly increased, the proportion of fermenting cells increased and soon no non-fermenting cells could be shown at all by plating. These results were obtained with several strains, but one or two strains behaved differently. In these cases, the count never rose to such high values as with the other strains, although one or two slight maxima occurred. Moreover, there were never more than a few red colonies to be obtained on plating.

These results were interpreted along the following lines. In the majority of cases, the production of a dulcitate-fermenting type after a few days leads to a sudden increase in growth since such cells would be able to use the dulcitate in the medium. There arises a conflict between this type and the non-fermenting type, and the former, with its advantage in its additional carbon source, soon predominates and eventually entirely excludes the latter. In the case where no large growth maxima occurred, the conflict was more even, now the one, now the other type gaining slightly.

So far we have considered the effect on enzyme production of the enzyme substrate. Of the instances recorded in the literature of substances unrelated to the substrate stimulating enzyme formation, perhaps the most interesting are those described by Jacoby (1916-18). In a series of papers dealing with the production of urease by bacteria, he claimed that the formation of an enzyme may be limited by substances in the medium which are needed by the cell as "bricks" (*Bausteine*) for the building up of the enzyme molecule. The carbohydrates, for example,

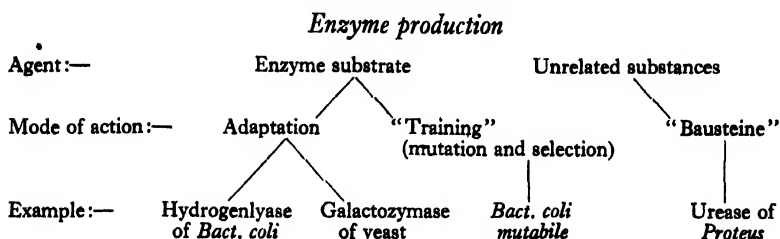
could be classed into four groups according to their efficacy in stimulating urease production in *Proteus vulgaris*, and from this Jacoby concluded that the group



was necessary for the formation of the enzyme molecule.

Although Jacoby's results can be criticized on the grounds of faulty experimental technique, the possibility nevertheless remains that enzyme production may be conditioned by substances which enter into the constitution of the enzyme molecule (see Passmore & Yudkin, 1937).

The types of enzyme production just discussed may be summarized as follows:



III. THE PERMANENCE OF THE VARIATION

An adaptive enzyme, arising from a direct interaction of the cell and the substrate, might be expected to be permanent only as far as the individual cells are concerned and to be lost when the cells are subcultured on media in which the substrate is absent. This is in fact the case. *Bact. coli* containing hydrogenlyase after growth in the presence of formate, loses it at once when subsequently grown in the absence of this substance. Yeast grown on galactose produces galactozymase, which is lost when subcultures are made into media not containing this sugar.

On the other hand, organisms possessing enzymes which arise as mutations would be likely to retain the enzyme in the absence of the substrate since mutations are generally permanent. Thus, after sufficiently long training, a strain of *Bact. typhosum* (Twort, 1907) and a strain of *Bact. paratyphosum* (Penfold, 1911), both variable to dulcitate, retained their dulcitate-fermenting properties after growing again in a dulcitate-free medium. If the period of training was shorter, slow reversion of Penfold's *Bact. paratyphosum* to the non-fermenting type did take place in the dulcitate-free medium, suggesting that a few of the original non-fermenting cells were still present in the culture.

IV. ENZYME FORMATION AND GROWTH

Several workers have at various times been engaged in attempts to show whether enzyme formation is linked with cell division. The experiments described in the literature which bear on this point are of three types. First, it has been stated,

notably by Euler and his co-workers, that the formation of invertase (see Euler, 1922) and galactozymase (Euler & Nilsson, 1925) by yeast cells require the presence of nitrogen-containing substances, i.e. substances without which growth is impossible. From this, Euler argued that "the increase in enzyme content is linked with the growth of the cells". However, although more invertase is formed in the presence of such compounds, quite a fair increase in the normal invertase content occurs in their absence (Kullberg, 1914). And in the case of galactozymase, Dienert (1900), Abderhalden (1926) and Stephenson & Yudkin (1936) were all able to obtain enzyme production in the presence of galactose alone.

The second type of experiment to test the association of growth and adaptation is that in which an attempt is made to secure adaptation in the presence of cell poisons. Dienert (1900) had claimed that galactozymase formation in yeast could occur in the presence of antiseptic doses of mercuric chloride or phenol. Euler & Nilsson (1925) claimed similar results with phenol, which were however criticized by Söhngen & Coolhaas (1926). The latter workers showed that in the conditions used by Euler & Nilsson, in which the phenol was present during the adaptation but not during the test for the fermentation of the galactose, a great increase in cell count takes place during the fermentation stage. They concluded therefore that there was still no evidence that adaptation could occur without the production of new cells. Euler & Jansson (1927) repeated their experiments, using phenol during the fermentation as well as during the adaptation, and found that, in this case, the galactose was not attacked, that is, there had been no production of galactozymase. Stephenson & Yudkin (1936) were also unable to obtain galactozymase formation in the presence of a sufficient concentration of antiseptic (such as mercuric chloride) to render the yeast culture completely sterile.

The third approach to the problem is that of cell counts. Söhngen & Coolhaas (1924) had found that the rate of production of galactozymase and the rate of increase in the cell count were exactly as would be expected if the production of the enzyme were associated with cell division. Stephenson & Yudkin (1936), however, by making viable and total cell counts during the adaptation, were able to obtain galactozymase production in the complete absence of cell division. Similar results with the hydrogenylase of *Bact. coli* had been obtained by Stephenson & Stickland (1933).

It is clear that from the point of view of deciding between the selection of cells containing a certain enzyme and its production as a direct action of the chemical environment on the cells themselves, it is important to show whether such a correlation between growth and adaptation exists. For if it were shown that enzyme production can occur without cell division, there can be no question of selection. However, if it were shown that enzyme production is normally associated with cell division, the selection hypothesis is by no means proved. The following considerations may make the position a little clearer.

It is true to say that in a growing culture of micro-organisms there is no essential difference between parent cells and daughter cells. A cell just before division, the parent cell, will have exactly the same chemical and enzymic make-up as the two daughter cells into which it divides on reproduction. A difference might of course

arise in the daughter cells if after division the latter are subjected to changes in environment. But, in the cases we are considering, the new environment existed for the original parent cells as well as the daughter cells.

To state, as for example did Söhnngen & Coolhaas, that enzyme formation occurs only in dividing cells and that only the new cells have the enzyme, seems to suggest that the enzyme suddenly appears at the actual moment of division. It is hardly conceivable that at this moment the cell is capable of being stimulated to enzyme formation and that this cannot occur at any other time. Of course, if the number of cells increases considerably during the time the enzyme is being formed, as did actually occur in the experiments of Söhnngen & Coolhaas, one might suppose that selection is at work; but, in fact, the question of deciding between chemical action and selection had not been formulated at the time. Cell division then can only be taken as an indication that the individual cells are growing—forming new protoplasm—since such cell growth is normally the prelude to cell division.

On the other hand, the demonstration in two cases (galactozymase in yeast and hydrogenylase in *Bact. coli*) that enzyme production may be dissociated from cell division definitely rules out any question of selection in these two instances. In both cases, the absence of division was quite fortuitous; there was nothing added to prevent cell division. Given suitable conditions there is no doubt that the majority of the cells would have divided. It would be interesting to obtain enzyme production in cells which, by being treated with cell poisons, have lost the power to divide. Although, as already mentioned, several attempts to obtain the formation of, for example, galactozymase, in conditions in which cell division has been rendered impossible have so far failed, there seems no *a priori* reason why it should not be possible. (Results suggestive of such a dissociation of viability and enzyme formation by the use of ultra-violet light in yeast producing galactozymase were obtained by Stephenson & Yudkin (1936).) However, even if this dissociation is conclusively obtained, its interpretation rests on the definition of growth and life in relation to micro-organisms. It might well be argued that cells incapable of division but capable of enzyme production are alive. If the main criterion of growth is an increase in complexity, then again organisms producing a new enzyme are growing. Only if enzyme production were found in cell-free preparations such as yeast juice would the question of adaptation and growth be settled.

It is interesting to compare the dissociation of cell division and enzyme production in the production of galactozymase and hydrogenylase with the examples of dissociation collected by Needham (1933). Many cases are quoted in which enzyme action may be dissociated from growth, to which may be added the effect of silver on washed suspensions of *Bact. coli* (Yudkin; unpublished observations). It was here found that cell division is completely inhibited by a silver concentration about one-hundredth of that necessary to inhibit completely succinic, lactic or glucose dehydrogenase. An example in the opposite sense is that of Holmes (1933) in which it was shown that glycolysis of a tissue culture (rat kidney) can be inhibited by 50 per cent by treatment with radium emanation without impairing the growth. In fact Doljanski (quoted by Needham, 1933) concludes from his studies of the

production of melanin by iris cells and of glycogen by liver cells, that "there is an antagonism between multiplication and the physiological action of the cell". One might suppose that the absence of cell division during enzyme production in the cases of galactozymase and hydrogenlyase supported this view. The experiments of Stephenson & Stickland (1933), however, have shown that growing cells producing hydrogenlyase divide as rapidly as cells producing no enzyme.

V. TELEOLOGY AND ENZYME PRODUCTION

Perhaps the most interesting fact in the study of enzyme variation in micro-organisms is that enzyme production is stimulated by the presence of the enzyme substrate. It is not surprising therefore that from the earliest worker in this field (Wortmann, 1882), almost up to the present day, "explanations" of enzyme formation as being determined by the need of the cell occur throughout the literature. We may quote a few of the more recent examples. In 1915, Kendall & Walker, maintaining that proteolytic enzymes were formed by *Proteus* only in the absence of sugar, state: "When the sugar is exhausted, the organism is forced to derive its enzyme from protein constituents and the enzyme is then formed to bring about the necessary changes in the protein to make it assimilable." Working also with proteolytic enzymes, Diehl (1919) says: "From our knowledge of vital processes in general, it seems more probable that the bacterial cells should be endowed with the ability to elaborate ferments than that it should be a storehouse for the many ferments of which it has need at one time or another; or even that it should form all the ferments within its power each time there is a need for any one of them." However, Quastel & Whetham (1925) have shown that *Bact. coli* possesses enzymes which oxidize a large number of compounds with which the organism normally never or rarely comes into contact. Diehl says further that his results "furnish evidence that bacterial enzymes are not preformed in the cell but are manufactured by the organism as the need for them arises". Lastly, we may quote the views of Karström (1930) on the production of disaccharidases by *Bact. coli* on all sugars except glucose: "Sehr wahrscheinlich scheint uns daher eine solche Deutung der Tatsachen dass die an Glucose gewöhnten Bakterien die Disaccharidasen überhaupt nicht produziert haben, weil sie diese Enzyme bei ihrem Wachstum nicht brauchten."

An interesting analogy to adaptive enzyme formation exists in the preparation of an inorganic catalyst, for which it would be difficult to invoke teleology. The preparation of a palladium catalyst is usually carried out by the reduction of one of its salts, e.g. the nitrate. This can be done in a variety of ways, all of which result in a preparation which can reduce methylene blue in the presence of formate (that is, which can act as "formic dehydrogenase"). If, however, it is required to prepare a catalyst which is capable of producing molecular hydrogen from formate (that is, capable of acting as "formic hydrogenlyase"), the reduction is effected with formic acid. The analogy with *Bact. coli* is very striking—the bacteria grown in any conditions contain formic dehydrogenase, but only when grown on formate do they contain formic hydrogenlyase. Although of course this analogy can readily be carried too far, it appears

sufficiently striking to warrant mentioning. In whatever way the phenomenon in the case of the inorganic catalyst will eventually be explained it will certainly not be along lines suggesting an almost conscious regulation in its formation.

Another argument which may be adduced against those who too readily adopt a teleological explanation of enzyme formation is that, in a great number of cases, the products of the action of the enzyme on the substrate are often as effective in stimulating enzyme production as the substrate itself. If an enzyme is formed in the presence of a substrate because the organism needs the products of enzyme action, then the presence of such products should prevent its formation. Where the substrate gives an increase of enzyme rather than an apparent formation *de novo*, as with yeast invertase and sucrose, the products, in this case glucose and fructose, should result in a decrease in the enzyme. Actually they produce as much increase as sucrose itself (Meisenheimer *et al.* 1913, 1914; Euler & Cramer, 1913). Further examples are given below.

VI. THE "MASS ACTION" THEORY OF ENZYME FORMATION

(1) *Enzyme formation by the enzyme substrate*

Apart from teleological "explanations" and statements that it is associated with the vital processes, there has been no general theory of enzyme production: one which could explain the large mass of accumulated data and which at the same time would be capable of being treated experimentally. The following is an attempt to construct such a theory.

In several instances, the production of the enzyme is relative and involves an increase in pre-existing enzyme, whereas in others there is apparently a production of new enzyme. It is possible that in these latter cases, there is in fact a little enzyme present but not sufficient to be measured by the technique adopted. If this is so, all examples of enzyme production are cases of increase in enzyme and none are instances of the formation of completely new enzyme.

Without attempting to postulate any definite compounds or definite sequence of events, it is clear that the adaptive enzyme is produced from a precursor or precursors. It does not matter for the purposes of this discussion whether such precursors are compounds of the enzyme with some inhibiting substances or whether they form part of the cell protoplasm normally concerned in some quite other process. In either case it is assumed that an equilibrium exists between such precursors and the formed enzyme. An immeasurably small amount of enzyme in untreated cells would mean that the equilibrium is on the side of the precursors. The combination of the enzyme with any substance would result in a disturbance of the equilibrium and more enzyme would be formed from precursor in order to restore it. Such a virtual removal of the enzyme could of course be effected by combination with its substrate.

We have here then a simple conception of the process by which enzyme production may be induced by the presence of the substrate of the enzyme. The addition of the substrate means that a part of the enzyme present is at any given

moment combined with the substrate and the restoration of the precursor-enzyme equilibrium involves the formation of more enzyme from precursor.

Viewed from the standpoint of this theory, the literature offers a number of facts which support it and which are otherwise difficult to explain. Moreover, many deductions are possible from the theory which are readily capable of being tested experimentally.

(2) *Enzyme formation by the products of the action of the enzyme*

It is generally assumed that enzyme action is reversible, although this is not always easily demonstrable. An enzyme must therefore be able to combine with the products as well as with the substrate. For example, a lipase, as well as adsorbing a fat, should adsorb the fatty acid, the glycerol, or both. According to the theory, then, we should expect that enzyme production would be stimulated not only by the substrate but by one or more of the products of enzyme action. A study of those instances in the literature where substances other than the substrate are effective in enzyme production reveals that they are very often just such products of enzyme action. A few examples may be quoted.

(1) The formation of diastase by *Aspergillus niger* is stimulated by starch or maltose but hardly at all by glycerol or sucrose (Funke, 1923).

(2) Melibiase or lactase of yeast are increased as well by galactose as by the sugar concerned (Dienert, 1900).

(3) Yeast invertase is increased by fructose or glucose as well as by sucrose (e.g. Euler & Cramer, 1913).

(4) Of several substances related chemically to tannic acid which were investigated by Knudson (1913), only gallic acid was able to effect tannase production in *Penicillium*.

(5) Oleic acid is as effective as triolein in the production of lipase in *Aspergillus niger*, whilst peptone and sucrose, or glycerol, are ineffective (Schenker, 1921).

The fact that glycerol does not stimulate lipase production in *A. niger* suggests an interesting speculation. According to the theory, such a result must mean that the glycerol does not combine with the enzyme. The synthetic action of lipase would then be entirely confined to "activating" the fatty acid in such a way that it can combine with unadsorbed glycerol. If the theory of enzyme formation here developed is correct, such results would be of interest from the point of view of the mechanism of enzyme action.

(3) *Enzyme formation by substances related to the substrate or to its products*

The substrate or the products of the action of the enzyme on the substrate are by no means the only substances which stimulate enzyme formation. Glucose is as effective as starch or maltose in diastase formation by *Aspergillus* (Funke, 1923) and mannose increases the invertase of yeast even more than sucrose or glucose (Euler & Cramer, 1914). Maltase in *Monilia sitophila* is produced when growth takes place on maltose or substances such as starch or dextrin which readily give maltose, but galactose, sucrose and trehalose are just as effective (Went, 1901). Such facts can be

explained by assuming that these related substances can combine with the enzyme. It is well known that substances chemically related to the substrate of an enzyme are often adsorbed at the enzyme and cause a competitive or non-competitive inhibition of the enzyme-substrate reaction. It would be of interest therefore to investigate these related substances concerned in enzyme formation to see whether they act as inhibitors. It is possible that such substances combine with the enzyme at some point other than that at which the substrate combines. They would then give rise to a production of enzyme without causing inhibition. Conversely, however, it would be expected that substances which cause an inhibition by combining with an adaptive enzyme would also stimulate its formation. It is known for example that mannose, which inhibits the action of yeast invertase, increases its production. Maltose, on the other hand, although it does not inhibit the action of invertase, is known to combine with it since it is able to remove the enzyme from adsorption on colloidal ferric hydroxide (Michaelis, 1921). It would be interesting to see whether maltose stimulates invertase production in yeast.

(4) *Deductions from the theory*

So far, no direct attempt has been made to test the theory, but it will be of interest to consider in what directions logical deductions from the theory would lead.

It has already been noted that one such deduction involves the investigation of the reversible inhibitors of the adaptive enzyme from the point of view of their ability to stimulate the formation of these enzymes. For example, it is known that galactose and arabinose act as competitive inhibitors in the action of yeast invertase on sucrose: i.e. both these monosaccharides can be adsorbed at the enzyme surface and can be replaced by sucrose. They should both therefore be effective in increasing yeast invertase.

In order that a substance inhibiting an enzyme reaction by combination with the enzyme shall act as a stimulus to enzyme production, two conditions must be fulfilled. First, the combination between enzyme and inhibitor must be reversible. The inhibition of enzyme reactions by metals is in many cases not reversible. If such metals do cause an increased production of enzyme, therefore, the enzyme formed could not be freed from the metal inhibitor and would be inactive.

The second condition is that the inhibitor should combine with the enzyme but not with the precursor. If the inhibitor is generally adsorbed by both enzyme and precursor, a disturbance of the precursor-enzyme equilibrium would not occur and no enzyme would be formed. This would seem to preclude the use of unspecific reagents such as metals in enzyme formation, even if the enzyme-metal combination was reversible.

The results of Katz (1898) are of interest in this respect. The diastase of *Aspergillus niger* can be precipitated from solution by tannic acid and the active enzyme can readily be regenerated from the precipitate by alcoholic extraction of the tannic acid. Katz claimed that the presence of tannic acid in the medium in

which the mould is grown resulted in a greatly increased production of diastase. This could be explained by the combination of the tannic acid with the enzyme. It has been shown, however (Yudkin, 1936), that the action of the tannic acid is to increase the growth of the fungus. The total amount of diastase is therefore greater in the mould grown in the presence of tannic acid, but the amount of enzyme per gram dry weight of mould is in fact found to be somewhat less than in the control. This result was not unexpected in view of the very unspecific combining powers of tannic acid.

Besides substances which inhibit enzyme reaction, other substances are known to be adsorbed by enzymes. Such a case is that of maltose and yeast invertase mentioned above. It would be of interest to study the effect of this sugar on the production of invertase by yeast.

Apart from yeast invertase, little work has been done with adaptive enzymes on their combination with substances other than the substrate. A systematic attempt to test the theory might well begin with the investigation of several substances from the point of view of their effect on, for example, galactozymase action. This could be done in the first place by testing a number of compounds for competitive inhibition and then for their effect on enzyme formation.

Quantitative studies, of which very few appear to have been made, should throw a great deal of light on the mechanism of enzyme formation. The amount of enzyme formed with varying concentrations of substrate has been studied in fair detail only with one enzyme—the invertase of *Penicillium* (Kertesz, 1929). The form of the curve relating substrate concentration and amount of enzyme produced is very much like an ordinary adsorption isotherm and suggests that the amount of enzyme formed is directly proportional to the amount of substrate adsorbed. This is in agreement with the theory. A second quantitative line of investigation would be a study of the effect of substrate concentration on the *rate* of enzyme formation. Such a study should give some insight into the nature of the precursor-enzyme reaction.

VII. SUMMARY

The increase in enzyme content in micro-organisms may be classified according to the type of substance responsible for the increase. First, as has been suggested by Jacoby, the substance may be necessary to the cell as a "brick" for the building up of the enzyme molecule. Secondly, the substance may be the substrate of the enzyme or some closely related compound. A substance in this class may act either directly on the individual cell or as a selecting agent during growth of cells in which the enzyme has appeared as a mutation. Examples of direct chemical interaction between cell and substrate (adaptation) are the hydrogenlyase of *Bact. coli* and the galactozymase of yeast; examples of mutation and selection occur amongst organisms of the *Bact. coli mutabile* type.

As would be expected, when organisms possessing a newly acquired enzyme are grown in the absence of the substrate, enzymes arising by adaptation are readily lost whilst enzymes arising by mutation and selection tend to be permanent.

It would also be expected that the former type of enzyme production would occur in the absence of cell division; for natural selection to operate, however, cell division is essential. Many unsatisfactory attempts have been made to show the dependence or otherwise of certain examples of enzyme production on cell division, such as the study of enzyme production in the presence or absence of suitable growth substances or of poisons. By counting experiments, however, it has been clearly shown that, in the two examples of adaptation quoted, enzyme production occurs in the absence of cell division.

Although enzyme production has been obtained in non-dividing (but untreated) cells, it has not yet been found possible to obtain it in cells which have been rendered non-viable, that is, incapable of dividing. It would be of interest if such poisoned cells would be found still able to produce the adaptive enzyme, but it is necessary to define carefully the meaning of living cell and growth in relation to micro-organisms before deciding on the dependence of enzyme production on cell viability or cell growth.

Apart from general statements of a teleological nature, the literature contains no general theory of adaptive enzyme production. The "mass-action" theory of enzyme formation here developed explains the majority of the known facts, is in contradiction with none of them, and allows several deductions to be made which are capable of being tested experimentally.

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THE GENETICS OF SEX IN LEPIDOPTERA

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I. SEX HORMONES

IN the Vertebrata the influence of hormones secreted by the gonads and other endocrine glands is too well known to need recapitulation, but in the Lepidoptera the state of affairs is very different and, if sex hormones exist, either they are intracellular and do not circulate in the body fluids or they are elaborated in one part and act upon another part of the same gonad. The evidence for this statement is afforded by (1) experimental castration, (2) transplantation of gonads, (3) gynandromorphs.

Oudemans (1899) removed the testes or ovaries from larvae of *Lymantria dispar* L. before the penultimate and final moults. Thirty out of sixty survived, but both males and females were unaltered in appearance and their sexual dimorphism was as pronounced as in intact moths. Their sexual instincts were unchanged; castrated males copulated and castrated females tried to lay eggs, but were only able to deposit the tuft of woolly hair, with which normal females cover their eggs. Kellogg (1904) performed similar experiments with the silk moth, *Bombyx mori* L., and again no modification of the secondary sexual characters resulted.

Meisenheimer (1907), meeting the objection that Oudemans's experiments were performed too late in larval life, castrated 600 larvae of *Lymantria dispar* and bred 186 moths. Castration before the second moult was fatal, but some of those operated on between the second and third, and third and fourth moults survived. In the moths the secondary sexual characters were unaffected. Even these experiments of Meisenheimer are not free from the objection that a sex hormone might have been present and acted before the removal of the gonads.

Castration followed by transplantation of the gonads of the opposite sex was done by Meisenheimer (1907), but this was equally without effect on the development of the secondary sexual characters, nor did removal of one gonad with substitution of the gonad of the opposite sex make any general alteration in the appearance of the moths, or cause any difference in the secondary sexual characters on the two sides.

Gynandromorphs, as Cockayne has pointed out (1916), afford still better evidence that there are no circulating hormones at any period of development. In male Lepidoptera there are, in addition to the gonads, two glandulae accessoriae, and in the female two cement glands and the spermatheca, which might conceivably secrete a hormone in addition to their other functions. Even in gynandromorphs, which are divided accurately into male and female halves externally, the internal organs may not show a corresponding division. A gynandromorph of *Amorpha populi*, for example, may have two ovaries, one or two cement glands, and a spermatheca, and neither testes nor glandulae accessoriae, but the absence of male organs does not prevent the full development of male secondary sexual characters on the one side, nor does the presence of female organs influence them in any way. Similarly female organs may be absent and male organs present in a gynandromorph which is male on one side and female on the other, and on neither side are the secondary sexual characters modified in any way. In these cases we have Meisenheimer's castration and transplantation experiment carried out by nature at the very earliest stage of development. In some gynandromorphs, perfectly divided into a male and female half, the gonad and accessory organs of both sexes may be present, but they exert no influence on the sexual characters of the opposite side, or indeed of those on their own side. In a gynandromorph of *Amorpha* hybrid *hybridus* Steph. which was dissected, the right half showed the secondary sexual characters of the male and the left side those of the female, but neither ovary nor testis was present, and one cement gland and the bursa copulatrix, both imperfectly formed, were the only internal genital organs present. To sum up—the vasa deferentia, vesiculae seminales, ductus ejaculatorius, glandulae accessoriae and testes may be absent in the male without preventing the secondary sexual characters from appearing, and oviducts, vagina, bursa copulatrix, cement glands, spermatheca, and ovaries may be absent in the female without producing any effect on the external appearance.

Wigglesworth (1936) has discovered that in *Rhodnius* (Hemiptera) the corpus allatum, situated in the head, elaborates a hormone, identical in both sexes, which circulates in the blood and in the male causes the glandulae accessoriae to complete

their development and in the female causes the ova to mature. It is not improbable that a similar hormone is secreted by the corpus allatum in Lepidoptera, for, if it is the same in both sexes, neither gynandromorphism nor experimental castration, with or without implantation of the gonads of the opposite sex, would interfere with its action and so reveal its existence. The experiment of Bytinski-Salz (1933), though not conclusive, suggests that such a hormone is present in Lepidoptera. He found that female pupae of the hybrid *Celerio gallii* ♂ × *Celerio euphorbiae* ♀ do not reach the imaginal stage, but, if their ovaries are transplanted into the male pupae, which develop normally, the ova become fully mature.

II. SEX DETERMINATION

(1) *Abnormal sex ratios and intersexes*

Before discussing the cause of abnormal sex ratios and intersexes it is necessary to say a few words about sex determination in Lepidoptera. The female in this Order is heterogametic for sex, having a *Y*-chromosome and only one *X*-chromosome, while the male is homogametic for sex and has two *X*-chromosomes.¹ The mere presence of one or two *X*-chromosomes is, however, insufficient to determine whether a moth shall be a female or a male. Goldschmidt by his work on *Lymantria dispar*, summarized in 1934, has proved that sex is determined by a balance between male and female factors. In *dispar* the factor for maleness is in the *X*-chromosome and a factor for femaleness is in the cytoplasm, and both these factors vary in valency in different races. There is also a factor for femaleness in the autosomes, and the breeding experiments of Goldschmidt and Schweitzer have proved that this too varies in valency in different races. When the male valency of the *X*-chromosome is sufficiently above the female valency of the cytoplasm, a female intersex is produced and an insect with its genetic constitution *XY* shows male characters. Similarly, when the male valency of the *X*-chromosomes is sufficiently below the female valency of the cytoplasm, a male intersex is produced and an insect with its genetic constitution *XX* shows female characters. The degree of intersexuality is dependent on the quantitative difference in these valencies, and in extreme cases complete sex reversal is brought about. Males transformed into females, however, almost always die in an early stage and are sterile even if they survive, but females transformed into males are viable and fertile, though in many cases they are less viable than normal males. In these all-male families, a female transformed into a male can only be recognized by the abnormal sex ratio, two females to one male, which results from a pairing with a normal female. Goldschmidt has also shown that in *L. dispar* the greater the difference in valencies the earlier the change of sex begins and, since the differentiation of various organs takes place at different periods of embryonic life, the greater will be the number of organs showing changes in structure towards that of the opposite sex and the more profound will be the changes in those differentiated earliest.

¹ *X* and *Y* are used whether the female is homogametic or heterogametic for sex, though *Z* and *W* are used in the latter case by some authors.

In the experiments, which proved that variation in the female valency of the autosomes exists, a female with strong female valency in the cytoplasm was chosen as proband, thus ensuring that all its descendants were uniformly strong in this respect, and introducing an *X*-chromosome with strong male valency and a strongly female autosomal factor (*T*). A weakly female autosomal factor (*t*) and an *X*-chromosome of weak male valency were introduced by using a weak male as the other parent. In the F_2 generation an eighth of the males were intersexual, and in the F_3 generation the ratios of normal to intersexual males were 1 : 1, 3 : 1, or 7 : 1, and all the males were intersexual when the *X*-chromosomes of both parents had a weak male valency and their autosomes were both weakly female (*tt*). These experiments have shown that all male intersexes, whose intersexuality is attributable to the *t* factor are homozygous for it and have both *X*-chromosomes of weak male valency while their cytoplasm is of strong female valency.

There can be little doubt that the secret of the abnormal sex ratios and of the occurrence of intersexes in primary hybrids depends in a similar way upon the fact that the valency of the *X*-chromosome differs in different species. In hybrids, if the valency of the *X*-chromosome of the male parent is very much greater than that of the female parent, the presence of even one *X*-chromosome may so preponderate over the influence of the autosomes and cytoplasm, that a male is produced where a female is expected, and whole broods may consist of males only, some with two *X*- and others with only one *X*-chromosome. If, however, the valency of the *X* of the male parent is rather less, but still considerably greater than that of the female parent, intersexes may be produced instead of females. As the valency of the *X*-chromosomes of the two species becomes nearer to equality, intersexes become rarer, but still occur in small numbers amongst the infertile females, which are unable to lay eggs. When the *X*-chromosomes are still more nearly equal in valency females are produced, which can lay eggs, but the eggs do not hatch, and in the next stage the females are partially fertile.

Thus the Lepidoptera follow the law enunciated by Haldane (1922), that if, in the offspring of a cross between species, one sex is rare, sterile, or absent, this is always the heterogametic sex.

Harrison classifies his Bistonine hybrids into the following six groups:

(1) Hybrids which produce all males, the male parent being phylogenetically the older species:

- Lycia hirtaria* ♂ × *Nyssia zonaria* ♀.
- Lycia hirtaria* ♂ × *Nyssia graecaria* ♀.
- Poecilopsis pomonaria* ♂ × *Nyssia zonaria* ♀.
- Poecilopsis isabellae* ♂ × *Nyssia zonaria* ♀.
- Poecilopsis lapponaria* ♂ × *Nyssia zonaria* ♀.

(2) Hybrids which produce males and intersexes instead of females:

- Lycia hirtaria* ♂ × *Poecilopsis rachelae* ♀.
- Poecilopsis rachelae* ♂ × *Nyssia zonaria* ♀.

(3) Hybrids which produce sterile females unable to lay eggs and occasional intersexes:

Poecilopsis pomonaria ♂ × *Poecilopsis lapponaria* ♀.

(4) Hybrids which produce sterile females unable to lay eggs:¹

Lycia hirtaria ♂ × *Poecilopsis lapponaria* ♀.

(5) Hybrids which produce females capable of laying eggs, which fail to hatch:

Lycia hirtaria ♂ × *Poecilopsis isabellae* ♀.

(6) Hybrids which produce both sexes in ordinary proportions with the females partially fertile:

Lycia hirtaria ♂ × *Poecilopsis pomonaria* ♀.

Poecilopsis pomonaria ♂ × *Poecilopsis isabellae* ♀.

Poecilopsis isabellae ♂ × *Poecilopsis pomonaria* ♀.

Only one secondary Bistonine hybrid has given all male broods, *Poecilopsis* (*pomonaria* ♂ × *lapponaria* ♀) ♂ × *Nyssia zonaria* ♀, and this is only to be expected, since in the moths, which should be female, the *X*-chromosome must be either that of *pomonaria* or *lapponaria*, and both are of sufficient valency to give all male broods in the primary hybrid with *zonaria* as the female parent.

According to Harrison, weakening of the valency of the *X*-chromosome may take place as a result of inbreeding for several generations, and hybrids, which usually produce nothing but males, may then produce a few females. He obtained this result with the following hybrids, the male parent in each case being from inbred stock:

P. pomonaria ♂ × *N. zonaria* ♀ (a few females)

P. pomonaria ♂ × *P. lapponaria* ♀ (a few females)

P. pomonaria ♂ × *N. zonaria* ♀ (7 females)

P. lapponaria ♂ × *N. zonaria* ♀ (3 females).

and from eggs of the last-named cross, given to me by Harrison, I also bred 1 female and 9 males (unrecorded).

Since sex is due to the balance between the *X*-chromosomes and either the cytoplasm or the autosomes, we might expect that when one cross produces nothing but males, the reverse cross would produce an excess of females or intersexes. The weak *X*-chromosome derived from the male parent would fail to balance the strong female valency of the cytoplasm or of the autosomes, half of them being of strong female valency derived from the female parent, so that some moths genetically males would be transformed into females or intersexes. Actually of the five Bistonine hybrids, which usually give nothing but males, the reverse hybrid has only been obtained in three cases, but in all three there is an excess of females. *Nyssia zonaria* ♂ × *Lycia hirtaria* ♀ gave 94 males to 200 females, *N. zonaria* ♂ × *Poecilopsis pomonaria* ♀ gave 44 males to 102 females, and, though the numbers are not stated, *N. zonaria* ♂ × *P. lapponaria* ♀ also gave an excess of females. In none of them were any intersexes bred.

¹ Eggs were obtained from a female of this hybrid in 1934.

Hybrids between *Ectropis bistortata* Goeze. and *E. crepuscularia* Bkh. give abnormal sex ratios, whether the univoltine or bivoltine race of the latter is used. In hybrids with *crepuscularia* as the male parent the ratio of males to females was 959 : 8 in 15 broods combined, and females were altogether absent from 13 of them. There were three exceptional broods not included in these figures, in which there was an approach to equality of the sexes, and in two of them the female parent came from Yorkshire and in one from Durham. In hybrids with *bistortata* as the male parent the ratio of males to females was 1185 : 1215 in 31 broods combined, the data being collected from the results published by Tutt (Riding & Bacot's experiments, 1906), Cockayne (1909), and Harrison (1923, 1927, 1932). Thus the male valency of the X-chromosomes is so much greater in *bistortata* that females are as a rule transformed into males. In the secondary hybrid *E. (crepuscularia* ♂ × *bistortata* ♀) ♂ × (*bistortata* ♂ × *crepuscularia* ♀) ♀ two broods combined gave a ratio of males to females of 176 : 86, in both of them the ratio being about 2 : 1. Other secondary or more complicated hybrids gave about equal numbers of the two sexes or else the number of males was about double that of females.

The hybrid *Smerinthus ocellatus* L. ♂ × *Amorpha populi* L. ♀ (hybrid *hybridus* Steph.) has been bred many times and as a rule these broods consist of males only, but in some there are a small number of intersexes. Standfuss (1907) records that in 51 broods he got 1200 moths, 2 per cent of which were intersexes, and Oberthür, quoted by Tutt (1902), in 22 broods got 500 moths with 2 per cent of intersexes. In North Africa *A. austati* Staud. replaces *populi*, and according to Austat (1902) the hybrid *S. ocellatus* ♂ × *austati* ♀ (hybrid *operosa* Standfuss), gives 86 per cent of males and 14 per cent of intersexes, half of which are more or less crippled. *S. atlanticus* Aust. represents *ocellatus* in North Africa, and in a brood of the hybrid *S. atlanticus* ♂ × *A. austati* ♀ there were 45 males and 5 females, but these reputed females were not critically examined and were probably intersexes, and in 7 broods of the hybrid *S. atlanticus* ♂ × *A. populi* ♀ there were 10 per cent of females.

The intersexes of hybrid *hybridus* show an approach to male structure in the antennae and are stouter in build than females of either parent species. Bacot (1898) says that the external genitalia show almost every gradation from those almost completely female to those almost completely male, and I have a long series of preparations showing the same state of affairs. In many of them there is a coarse mosaic of male and female chitin; in some male valves and penis are well-formed and the only female structure is a little piece of ovipositor replacing part of the uncus, while in others the only male structure is a piece of uncus replacing part of the ovipositor. Roepke (1908-9) examined 22 males of this hybrid and found no female structures and the gonads were testes with degenerate spermatocytes or spermatozoa, but the one intersex had a mosaic of male and female parts in the external genitalia and aborted ovaries. He examined five so-called females of the hybrid *operosa*, and all showed a mixture of male and female structures, forming a coarse mosaic, in the external genitalia, and all had monstrous and deformed ovaries containing degenerate oogonia or oocytes.

Other hybrids have given abnormal sex ratios, but the numbers bred are too few for any definite conclusions to be drawn.

In different races the valency of the *X*-chromosome may show a constant difference as the sex ratios of Goldschmidt's crosses of *Lymantria dispar* from various continental localities proves. The same kind of difference has been demonstrated by Harrison (1919) in the English and Scottish races of *Lycia hirtaria*. The hybrid *pilzii* (*P. pomonaria* ♂ × *L. hirtaria* ♀ from England) gave an equal number of males and females, but, when the *hirtaria* females came from Scotland, the ratio of males to females was 190 : 14, and a similar difference in the sex ratio occurred when the hybrid *pilzii* was crossed with female *hirtaria* from England and Scotland respectively, the former cross giving approximately equal numbers of the two sexes and the latter a great preponderance of males. *Hirtaria* from the continent resemble those from Scotland in their potency, for Oberthür (1897, 1900) got a large number of males of the hybrid *pomonaria* ♂ × *hirtaria* ♀ and only bred 6 females, while Meisenheimer (1924) bred 300 males and no females in 1917, and 1000 males and 6 females in 1918.

Evidence that the valency of the *X* may be reduced in individuals of the same species and even of the same race by means of inbreeding has been brought forward already in the case of some of Harrison's Bistonine hybrids. The following is another example of reduction of the valency of the *X* attributed by Harrison (1919) to the same cause. Inbreeding *Cosymbia* (*Zonosoma*) *orbicularia*, which produces two or three broods a year, he had an equality of the sexes from 1906 to 1910, but in 1910 there was a loss of vigour and sexual instinct, and an excess of females began to appear. In 1911 several broods were wholly female, while in others there were still a few males, but in 1912 all the broods were entirely female.

(2) *Intersexes obtained by crossing a bisexual and a parthenogenetic race*

Solenobia triquetrella Dbld. (Tineidae) has a bisexual race with a winged male and a female with rudimentary wings, and a parthenogenetic race with a larger female with rudimentary wings. The former race is very local, but the latter has a wide range. The usual result of crossing a male of the bisexual race with a female of the parthenogenetic one is a brood consisting of females only, but Seiler (1927) has found that in some cases half the broods are intersexes, while the remainder closely resemble males and females. In some of the intersexes the greater part of the insect is of one sex and only a small part, sometimes conspicuous and sometimes inconspicuous, is of the other sex. In others the male and female parts are approximately equal in amount. One part, such as an antenna, may be male, and another part, such as a wing, may be female or vice versa. The gonads may be testes or ovaries or ovotestes, and in the ovotestes one part may be completely male and another part female or the whole organ may contain an intimate mixture of male and female cells.

The bisexual race is diploid with 60 chromosomes in the somatic cells and 30 in the gametes, but the parthenogenetic race is tetraploid with 120 chromosomes in the somatic cells and 60 in the ova. The cross results in a triploid zygote with 90

chromosomes. In some cases the haploid sperm does not conjugate with the diploid egg nucleus and cells are formed with haploid and diploid numbers. In other cases the egg nuclei conjugate with one another and tetraploid cells are formed, and finally there may be repeated conjugations resulting in cells with 120, 180, and 240 chromosomes.

Thus in a single moth there may be haploid, diploid, triploid, tetraploid (perhaps pentaploid), sexaploid, and octoploid chromosome numbers, but the triploid is the commonest. Presumably the haploid and triploid cells are male and the diploid and tetraploid are female.

(3) *Triploid intersexes*

A number of secondary hybrids are known, which produce broods consisting half of males and half of intersexes or of a mixture of females and intersexes. Standfuss (1900, 1914) has shown that of the hybrid *Saturnia* (*pavonia* ♂ × *pyri* ♀) ♂ × *pyri* ♀ 5 out of 8 moths bred were intersexes, of the hybrid *S.* (*pavonia* ♂ × *pyri* ♀) ♂ × *pavonia* ♀ 12 out of 54 moths bred were intersexes, of the hybrid *S.* (*pavonia* ♂ × *spini* ♀) ♂ × *pavonia* ♀ 10 out of 207 moths bred were intersexes, and of the hybrid *S.* (*pyri* ♂ × *pavonia* ♀) ♂ × *pavonia* ♀ 42 males, 37 intersexes, and 1 female were bred. The intersexes showed a coarse mosaic of male and female characters in the wings and external genitalia, and their gonads were ovaries with only a few ill-formed eggs in them. It has been proved cytologically that *pavonia* has 29 and *pyri* 30 chromosomes, and that there is little or no conjugation of chromosomes in the primary hybrid, so that the sperm is diploid and the zygote of the secondary hybrid is triploid.

Harrison (1916 b) hybridized *Poecilopsis pomonaria* ♂ and (*Lycia hirtaria* ♂ × *P. pomonaria* ♀) ♀ obtaining males and intersexes, but no females. Meisenheimer (1924) bred a much larger number of secondary hybrids, and from the cross (*L. hirtaria* ♂ × *P. pomonaria* ♀) ♂ × *L. hirtaria* ♀ obtained 129 males and 42 intersexes, from (*P. pomonaria* ♂ × *L. hirtaria* ♀) ♂ × *P. pomonaria* ♀ 19 males and 8 intersexes, and from (*L. hirtaria* ♂ × *P. pomonaria* ♀) ♂ × *P. pomonaria* ♀ 5 males and 6 intersexes.

The first of Meisenheimer's three secondary hybrids gave females with normal external genitalia, some intersexes with a piece of rudimentary uncus replacing the tip of the ovipositor or with a more or less complete uncus, and transitional forms between these almost completely female ones to those, which had the external genitalia almost completely male with well-formed penis and valves. Internally some had ovaries with few or no ova and more or less imperfect female accessory organs, others had ovaries and very aborted female organs with the addition of male accessory glands, others had one ovary and one testis, each with the accessory organs proper to its sex, but distorted and ill-developed. One had two testes and a very much aborted ovary as well, and some had two testes with both male and female accessory organs or with only male accessory organs. The other secondary hybrids were very similar, though one (*pomonaria* ♂ × *hirtaria* ♀) ♂ × *pomonaria* ♀ had two ovaries and two testes, all rudimentary, and both male and female accessory organs.

Externally the intersexes were much more like females than males, but in many of them part of an antenna or other structure was entirely male.

The diploid number in *hirtaria* is 28 and in *pomonaria* 100, but the number in the hybrid may be less than that expected, assuming that each *hirtaria* chromosome conjugates with a *pomonaria* chromosome, and probably the large chromosome of *hirtaria* conjugates with more than one of the small ones of *pomonaria*. Kosminsky (1924) subjected larvae of *Lymantria dispar* to a high temperature, 30–35° C., and obtained small moths, from which he bred in the next generation 12 intersexes out of 43 moths in 2 broods, and pairing these he got 7 intersexes again in 2 out of 11 broods. One intersex was male on the left and female on the right side externally and female internally, while the others were males with a mosaic of male and female characters in the wings, antennae and external genitalia. He found that in the larvae the spermatocytes, instead of having the haploid number of 31 chromosomes, had 46 to 58. Spermatozoa with the extra number of chromosomes conjugating with eggs with the normal haploid number would give insects approaching triploids. Kosminsky thinks that this triploidy was responsible for the production of the intersexes or mosaic gynandromorphs. Goldschmidt, however, thinks Kosminsky has misinterpreted his results, one of his reasons being that intersexual males instead of intersexual females were bred.

In these triploids there are either 3 X , or 2 X and 1 Y , to three sets of autosomes, or, if conjugation of some autosomes takes place, there will be less than three but more than two sets of autosomes to 3 X or to 2 X and 1 Y . Since sex depends on the balance between the X -chromosomes and the autosomes, a ratio of X to sets of autosomes of 1 : 1 giving a male and of 1 : 2 a female in the case of a pure species, triploids with 3 X and three sets of autosomes have the 1 : 1 ratio which produces a male, but those with 2 X and three sets of autosomes have a ratio of 1 : 1.5, midway between male and female, and so are intersexes. Further complications are, however, introduced by the inability of some of the autosomes in hybrids to conjugate, and the number of these is not constant, and also by the different valency of the X -chromosomes. Harrison (1919) does not accept this view as a complete explanation of triploid intersexes, but attributes their intersexuality to mitotic irregularities. He says that there is no true reduction division in the primary hybrid, so that all the gametes in either sex contain two sex chromosomes and the zygote in the secondary hybrid therefore contains three. If its constitution is $XX+X$, it is wholly male, whatever happens, but, if it is $XY+X$, it is inclined to be female. Abnormal mitoses are liable to occur and to affect the sex-chromosomes as well as the autosomes. A Y may go undivided to one pole giving daughter cells XX and XXY , or an X and a Y may so interfere with one another that both are lost and the daughter cells are XX and XY . Thus there may be neighbouring cells of male type $X+X$ or of female possibilities XY and $XY+X$. Since such mitotic irregularities may take place at any stage of development, there may be a mosaic of equal portions of both sexes or the insect may be almost wholly female. Mitotic irregularities may also account for intersexes in primary hybrids. If many chromosomes fail to conjugate, irregular cell divisions may occur and many of the unpaired

chromosomes may pass to one cell and few to the other. This might upset the balance between sex-chromosomes and autosomes, and make one cell male and the other female, and from each cell well-defined male and female parts might arise in the hybrid moth. On Harrison's view both primary and secondary hybrids showing mixed male and female parts should be regarded as mosaic gynandromorphs rather than as intersexes, and the triploid *dispar* and possibly the Lycaenid intersexes would also fall into this group.

On the other hand many of the intersexes of *Lymantria dispar*, in which the chromosomes are normal, show as coarse a mosaic of male and female parts as the triploid intersexes, although the chromosomes are alike in every cell.

(4) *Intersexes in the Lycaenidae*

A peculiar form of intersex is found in several species of Lycaenidae, *Lysandra coridon* Poda., *L. bellargus* Rott., *Plebeius aegon* Schiff., *P. pseudaeon* Butl., and *P. armoricana* Oberth. In all of them the normal male is blue and larger than the female, which is brown with some blue scales near the base of the wings. The intersexes are almost always like normal females on one side, while on the other there are areas more or less thickly covered with blue scales and androconia, the battledore scent scales peculiar to the male, and in *coridon* there are long blue scales shorter and broader than the male hair scales. There is also reduction in the size of the wings on the intersexual side proportional to the number of male scales present. In some cases both sides have these male characters. The underside is almost always of female coloration. The external and internal genitalia are female, but may be imperfectly developed in *coridon* and *aegon*, the only two species examined, and in *aegon* the ovaries are often small and contain abnormally large or abnormally small ova (Cockayne, 1916, 1922).

The behaviour of the intersexes is like that of females and fertile eggs of *coridon* and *aegon* have been obtained, but the larvae either failed to hatch or died young, probably because of the difficulty of rearing these Lycaenidae rather than to lack of vitality.

These intersexes all have a restricted range and vary in numbers in different localities. In one colony of *aegon* the ratio of intersexes to females was 3 : 405 and in another 31 : 340.

Where intersexes of *aegon* and *coridon* are most common, there is a great excess of females, and in the case of *coridon* there are also a large number of aberrant forms, many of which are asymmetrical. The excess of females and aberrant forms is probably due to the same cause as the intersexuality, and this may be a triploid condition of the cells. Cytological examination is much needed to throw light on it. These intersexes are particularly interesting, because they occur regularly in a wild state. A summary of our knowledge of them has been published by Cockayne (1926-7).

(5) *Unisexual families*

Doncaster (1913, 1914) obtained a strain of *Abraxas grossulariata* L., originating from a cross between a wild female and a *lacticolor* male, which produced some families consisting of females only, some in which females greatly outnumbered males, and others in which there were equal numbers of both sexes. The tendency to produce unisexual families or families with very few males in them was transmitted for six generations by direct descent, with three exceptions, even when the male parent was unrelated.

All the females of this strain, whether they belonged to unisexual or bisexual families, had a diploid chromosome number of 55 instead of 56, but the males had the usual number of 56. In two families with a great excess of females a large majority of the eggs received 27 and of the second polar bodies 28 chromosomes, but in bisexual families the number of eggs with 27 and 28 chromosomes was about equal. The males always had 28 chromosomes in both primary and secondary spermatocytes.

Doncaster thinks that the chromosome number 55 and the tendency to produce unisexual families may be independent of one another. He believes that a *Y*-chromosome is missing in females with a diploid number 55, and that the eggs contain either 27 chromosomes with neither an *X* nor a *Y*, or 28 with an *X*. In the females which produce unisexual families, all the eggs have only 27 and, conjugating with spermatozoa with 28, give rise to females with 55 chromosomes. The few males present in some families would be accounted for by the exceptional cases, in which the egg received 28 and the second polar body 27 chromosomes. In females giving rise to bisexual families the eggs and second polar body received 27 or 28 chromosomes with equal frequency. Since the gene for *lacticolor* is in the *X*-chromosome, *lacticolor* would appear in some unisexual families and not in others according to the genetic constitution of the male parent, and the results seem to be in accord with expectation. The liability to produce unisexual families appears to be determined by a gene, which is dominant or nearly so, and sex-limited, because there are no males to transmit it. This gene causes the passage of the *X*-chromosome to the second polar body during the maturation of all or nearly all of the eggs.

Seiler (1923) has shown that unisexual families with no males in them occur in the Tineid moth, *Talaeporia tubulosa*, Retz., and are due to non-disjunction of the *X*-chromosome during spermatogenesis. In this species the male has 60 chromosomes ($58 + XX$) and the female 59 ($58 + X$), the *Y* being absent. Seiler observed that during spermatogenesis the two *X*-chromosomes might remain united and be lost, so that spermatozoa with 29 chromosomes, containing no *X*, were formed. These, fertilizing eggs with 30 chromosomes, gave rise to females with 59 ($58 + X$), but, fertilizing eggs with 29, they gave rise to exceptional females with 58 chromosomes, with no *X*. Such exceptional females fertilized by males with normal sperm produced unisexual families consisting only of females. Embryos with 60, 59, and 58 chromosomes were seen, but although spermatozoa with 2 *X* due to non-

disjunction might be expected to produce males with 3 X , no embryos with 61 chromosomes were seen, and it is probable that they fail to develop.

Seiler compares the state of affairs in *Talaeporia* with that in *Drosophila*, in which non-disjunction was found by Bridges. In *Drosophila*, in which the male is heterogametic, a zygote with no X is not viable, whereas in *Talaeporia* it is viable, though whether a moth so constituted can produce viable offspring is not yet settled. In *Drosophila* a zygote with one X is viable but infertile, whereas in *Talaeporia* it is in all respects normal.

Unisexual families have been recorded in *Acraea encedon* L. by Lamborn (1911, 1914) and in *Hypolimnas bolina* L., by Simmonds (1923, 1926, 1928, 1930). Lamborn obtained three generations of all female families by crossing the females with males from bisexual families. Simmonds has bred many families of *Hypolimnas bolina* consisting of females only, obtaining his eggs from wild females captured in the Fiji Islands, Viti Levu, Kandavu, and Vanua Levu, where a great excess of females has been noticed since 1882 and was probably present long before this date. In the other islands and throughout the rest of its range the two sexes are found in approximately equal numbers. He has found that the percentage of fertile eggs is much lower in unisexual than bisexual families, and that some eggs change colour, but fail to hatch, while others never change colour at all. In one case about a third of the eggs hatched, and of the remainder half developed, but did not hatch, and half never developed at all. Ford has suggested that there is a sex-limited lethal gene, which kills the male eggs, but further investigation, especially into the cytology, is needed before this hypothesis can be proved or disproved.

The explanation is not improbable, for Goldschmidt and his co-workers (1934) have found a dominant gene, which kills all or almost all males of *Lymantria dispar* of a particular genetic constitution. If one or both parents are homozygous for this gene, all female families or families with only an occasional male are produced. If both parents are heterozygous for it, the ratio is four females to one male, and, if only one parent is heterozygous, the ratio is two females to one male. Other abnormal sex ratios, such as eight females to one male, four to one, or two to one, are produced, if some males of a brood are susceptible to its action and others are not. Varying ratios are then caused by the different genetic constitution of the males making them susceptible or resistant to the lethal action of the gene, and by the presence of the lethal gene itself in the homozygous or heterozygous condition in one or both parents.

III. SEX-LINKED INHERITANCE

Although sex-linked inheritance was first demonstrated by Doncaster in *Abraxas grossulariata* L., in which ab. *dohrnii* Koenig., better known as ab. *lacticolor* Raynor, is determined by a recessive gene in the X -chromosome, such characters appear to be rare in the Lepidoptera. Harrison (1920) has shown that the banded ab. *latifasciata* Vrbdt. of *Oporinia autumnata* Bork. is a sex-linked recessive, Kühn & Henke (1935) have described a sex-linked character in the meal moth, *Ephestia*

kuhniella Zeller, and Tanaka (1922, 1924, 1925) has found that a semi-transparent appearance of the skin in the larva of the silkmoth, *Bombyx mori* L. is determined by a gene in the X-chromosome. A sex-linked dominant gene causing melanism in *Lymantria monacha* has been described by Goldschmidt (1934).

IV. SEX-LIMITED INHERITANCE

(1) *Sex-limited characters in Rhopalocera*

In various genera of butterflies there are dominant characters, which are only found in the females, but can be transmitted by either sex. *Argynnis paphia* L., the silver-washed fritillary, has a dark greenish female, ab. *valesina* Esp., which is absent in some places, more or less rare in others, and in the eastern part of its range replaces the normal form altogether. Most species of *Colias*, clouded yellow, have a white form of female, which is usually rarer than the normal yellow or orange one. Lastly some species of *Papilio*, swallow-tails, have one or more female forms differing both in colour and pattern from the males in addition to a male-like form. Goldschmidt & Fischer (1922) in the case of *Argynnis paphia*, and Gerould (1911, 1923) in the case of *Colias philodice* Godt. have shown that the sex-limited form of female is determined by an autosomal dominant gene. Fryer (1913) has proved that one sex-limited form of *Papilio polytes* L. is determined in the same way, and that the other sex-limited form is due to an additional modifying fact gene. Jacobson's experiments, recorded by de Meijere (1909), indicate that the various sex-limited female forms of *Papilio memnon* L., one of which has tails though the male is tailless, are determined in a similar way.

In *Colias philodice* Gerould originally thought that white was dominant in the female and recessive in the male, and that no white males appeared because a white-bearing spermatozoon could not fertilize a white-bearing ovum. As a result of further breeding he showed that males homozygous for white resembled normal males. Fischer's experiments with *Argynnis paphia* have also proved that males homozygous for the *valesina* coloration are like normal males, and the existence of a race, in which all females are *valesina* and presumably all males are homozygous for the character, shows that the condition is not lethal in the male. Fryer's explanation (1913) was put forward before sex-linkage was fully understood and is not satisfactory. Goldschmidt calls these sex-controlled characters and considers that they are unable to appear in the male in the same way as ordinary female secondary sexual characters, such as winglessness in some of the *Bistoninae*, but this simple explanation does not account for males with *valesina* coloration.

Cockayne (1932) offers an explanation, which gives theoretical results in accord with those obtained by actual breeding. He thinks that all these sex-limited forms are determined by an autosomal dominant gene, which only produces a visible effect in conjunction with a gene in the Y-chromosome, and that this activating gene is present in every Y-chromosome.

Fischer (1929-30) has collected records of a few male *valesina*. If these are true *valesina* and not due to scale defects, they can be accounted for by non-disjunction

and would be constituted XXY . The activating gene in the Y in conjunction with the dominant gene in the autosome would produce the *valesina* coloration and the presence of two X -chromosomes would make the insect male.

Pieris napi L. has an arctic and alpine race, in which the female, *bryoniae* O., is heavily dusted with black scales along the nervures, and by some this race is considered to be specifically distinct from *napi*. Main (1908, 1912) crossed males of this race with English *napi* females and obtained normal *napi* females. On the other hand, by crossing English *napi* males with *bryoniae* females he obtained females, all of which were *bryoniae*. Similar results were also got by Fischer (1925). Thus the *bryoniae* character is transmitted by all the females to all their daughters, but is not transmitted by the males. The simplest explanation is that it is determined by a gene in the Y -chromosome.

(2) Sex-limited hereditary cancer in lepidopterous larvae

In 1911 Federley recorded the death of all the males of a brood of *Pygaera pigra* from a disease of the haemolymph. In a further publication (1936) he says that the disease has proved to be a malignant tumour, which affects any tissues except those derived from endoderm. The larvae usually die at an early stage, but may survive until the last instar. The tumour varies much in histological characters and some cells are polyploid, but it was impossible to determine whether the majority were diploid or triploid.

In three broods of *pigra* descendants of the females of the original brood, out of 471 larvae, 84 male larvae died of malignant tumours, and 157 female moths were bred, but no males. Some larvae of both sexes died of disease of the alimentary tract, and some were killed for examination. A similar result was obtained by crossing one of the females with a male *P. curtula*; 44 male larvae died from tumours and 69 female hybrids were bred, but again no males.

The disease was transmitted by all the females to all their sons. The gene cannot be in an autosome and be controlled by sex, because, even if dominant, only half the females would transmit it and only half their sons would be affected. It cannot be in the Y -chromosome because this remains in the female line, nor can it be in an X -chromosome, because, if so, it must be dominant and females would be affected.

Federley says that normally in *Pygaera* no true polar bodies are formed, but the three polar nuclei fuse and mitotic divisions take place, but these soon come to an end and the cells perish. He suggests that in this strain a mutation has occurred in the Y -chromosome, which stimulates mitosis, and that the gene is recessive and is inhibited by the presence of an X -chromosome. In the females the triploid polocyte is XXY and though the Y carries the gene the two X -chromosomes prevent its action; and the polocyte soon degenerates. In the male, however, the triploid polocyte is XY and the one X -chromosome cannot prevent the action of the gene present in the two Y -chromosomes. The polocyte therefore develops very rapidly and forms the fatal polymorphic tumours in all the male larvae. The gene, being present in every Y -chromosome, is transmitted by every female.

V. GYNANDROMORPHISM

A number of hypotheses have been put forward to explain the origin of gynandromorphs, but only the more modern ones need be considered.

(1) A delay in the passage of the spermatozoon after entering the egg, so that the egg has begun to divide before the sperm nucleus reaches it, and only one-half is fertilized (Boveri, 1888).

(2) Only one polar body is extruded; a second maturation division takes place, and of the two ootids so formed one is fertilized and gives rise to female parts, while the other, unfertilized, gives rise to male parts (Whiting & Whiting, 1927).

(3) More than one spermatozoon enters the egg, and while one fertilizes it and forms female parts, the male parts are formed by the development of one or more of the others (Morgan, 1905).

(4) An *X*-chromosome is eliminated at the first division of the fertilized ovum, or at some subsequent cell division (Morgan, 1907).

(5) By non-disjunction an *XXX* individual is produced, and at the first or some very early embryonic division one of these becomes separated and passes to one cell, while the other two pass to the other cell. In *Drosophila* male parts would arise from the former and female parts from the latter, but in *Lepidoptera* the reverse would be the case (Morgan & Bridges, 1919).

(6) From a binucleate ovum, each nucleus fertilized by a different spermatozoon. In *Lepidoptera* one nucleus would have an *X*- and the other a *Y*-chromosome (Doncaster, 1914).

(7) Shock occurring just as the pupa is casting its larval skin (Poulton, 1926).

(1) On Boveri's hypothesis a spermatozoon after entering an egg may be delayed so long that the egg has begun to divide before the sperm nucleus reaches it. The sperm nucleus unites with one half, while the other remains unfertilized. His explanation was intended to apply to the honey-bee, *Apis mellifica*, in which the males are produced from unfertilized eggs and are haploid, while females are produced from fertilized eggs and are diploid. Morgan considers that the available evidence is against Boveri's view even in the honey-bee.

In *Lepidoptera*, if the egg had a *Y*-chromosome, the unfertilized half, having only a *Y* would be female, and the fertilized half, having an *X* and a *Y*, would also be female. On the other hand, if the egg had an *X*-chromosome, the unfertilized half would have one *X* and one set of autosomes and would be male, while the other half would have two *X*-chromosomes and two sets of autosomes and would also be male, there being the same balance between *X*-chromosomes and autosomes in both halves. It does not seem possible that in *Lepidoptera* gynandromorphs can arise in this way.

(2) In *Habrobracon*, a parasitic ichneumonid fly, males arise, as in *Apis*, from unfertilized eggs and females from those which are fertilized. Gynandromorphs of known parentage, some showing segregation of somatic characters, have been bred in considerable numbers by Whiting & Whiting (1927). They assume that the first polar body is extruded and divides, and that there is then a subsequent division

and would be constituted XXY . The activating gene in the Y in conjunction with the dominant gene in the autosome would produce the *valesina* coloration and the presence of two X -chromosomes would make the insect male.

Pieris napi L. has an arctic and alpine race, in which the female, *bryoniae* O., is heavily dusted with black scales along the nervures, and by some this race is considered to be specifically distinct from *napi*. Main (1908, 1912) crossed males of this race with English *napi* females and obtained normal *napi* females. On the other hand, by crossing English *napi* males with *bryoniae* females he obtained females, all of which were *bryoniae*. Similar results were also got by Fischer (1925). Thus the *bryoniae* character is transmitted by all the females to all their daughters, but is not transmitted by the males. The simplest explanation is that it is determined by a gene in the Y -chromosome.

(2) *Sex-limited hereditary cancer in lepidopterous larvae*

In 1911 Federley recorded the death of all the males of a brood of *Pygaera pigra* from a disease of the haemolymph. In a further publication (1936) he says that the disease has proved to be a malignant tumour, which affects any tissues except those derived from endoderm. The larvae usually die at an early stage, but may survive until the last instar. The tumour varies much in histological characters and some cells are polyploid, but it was impossible to determine whether the majority were diploid or triploid.

In three broods of *pigra* descendants of the females of the original brood, out of 471 larvae, 84 male larvae died of malignant tumours, and 157 female moths were bred, but no males. Some larvae of both sexes died of disease of the alimentary tract, and some were killed for examination. A similar result was obtained by crossing one of the females with a male *P. curtula*; 44 male larvae died from tumours and 69 female hybrids were bred, but again no males.

The disease was transmitted by all the females to all their sons. The gene cannot be in an autosome and be controlled by sex, because, even if dominant, only half the females would transmit it and only half their sons would be affected. It cannot be in the Y -chromosome because this remains in the female line, nor can it be in an X -chromosome, because, if so, it must be dominant and females would be affected.

Federley says that normally in *Pygaera* no true polar bodies are formed, but the three polar nuclei fuse and mitotic divisions take place, but these soon come to an end and the cells perish. He suggests that in this strain a mutation has occurred in the Y -chromosome, which stimulates mitosis, and that the gene is recessive and is inhibited by the presence of an X -chromosome. In the females the triploid polocyte is XXY and though the Y carries the gene the two X -chromosomes prevent its action, and the polocyte soon degenerates. In the male, however, the triploid polocyte is XY and the one X -chromosome cannot prevent the action of the gene present in the two Y -chromosomes. The polocyte therefore develops very rapidly and forms the fatal polymorphic tumours in all the male larvae. The gene, being present in every Y -chromosome, is transmitted by every female.

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giving rise to two ootids, one of which is fertilized, while the other is unfertilized. The fertilized ootid produces the female parts of the gynandromorphs, being diploid, and the unfertilized ootid produces the male parts, being haploid.

(3) Morgan (1905) suggested that gynandromorphism might be produced, if two or more spermatozoa entered an egg, and while one united with the ovum, the other developed independently, or, if there were two additional spermatozoa, these fused and formed the rest of the insect. There is no evidence that any lepidopterous gynandromorph has arisen in this way, but Harrison (1916 *a*) has described a mosaic, which can only be explained on this hypothesis. He bred a hybrid thorn moth, *Ennomos subsignaria* ♂ × *E. quercinaria* ♀, of which the left side was male and showed a combination of the specific characters of both parents, while the right side, also male, showed only the paternal characters and was pure *subsignaria*. The ovum must have contained an *X*-chromosome and being fertilized by an *X*-bearing spermatozoon was male. Having received an *X*-chromosome and autosomes from each parent it had the usual hybrid characters. This accounts for the left side of the insect. The right side, showing purely paternal characters, must have developed from a single spermatozoon, which with one *X* and one set of autosomes would produce male cells, or by the fusion of two spermatozoa, which with two *X*-chromosomes and two sets of autosomes, would also produce male cells. Harrison prefers the former explanation of the right side and Morgan the latter. Had the insect arisen from a binucleate ovum, both sides must have shown *quercinaria* characters.

If the ovum had contained a *Y*-chromosome instead of an *X*-chromosome, this insect would have been a gynandromorph, with female hybrid characters on the left side and male *subsignaria* characters on the right side.

Gynandromorphism produced in this way could only be proved in the case of a hybrid, and comparatively few hybrid gynandromorphs are available for study. All that I am aware of show both maternal and paternal characters in both male and female parts. Tutt (1902) describes a hybrid *Smerinthus ocellatus* ♂ × *Amorpha populi* ♀, which, he says, had the characters of *ocellatus* on the right side, which was male, and of *populi* on the left side, which was female. The figure given by Briggs (1881) shows that the characters of both species were present on both sides, and it differs in no way from other known gynandromorphs of this hybrid.

(4) Morgan & Bridges (1919) give conclusive proof that the majority of gynandromorphs of *Drosophila* arise by the elimination of an *X*-chromosome either at the first cleavage division or at some subsequent cell division. Proof was greatly facilitated by the large number of sex-linked recessive characters in this species and by the small number of chromosomes. By breeding from parents of known genetic constitution they were able to show that an *X*-chromosome had been eliminated to produce the male parts, and that the *X* eliminated was as often that derived from the father as from the mother. In some cases they were able to confirm the loss of an *X*-chromosome in the male part by cytological examination.

In Lepidoptera, however, few sex-linked characters are known and the chromosome number is usually high, so that it is difficult to prove that elimination of an *X*-chromosome has taken place. Fortunately, Doncaster bred two gynandromorphs

of *Abraxas grossulariata*, of which the pedigrees were known. The first arose from a cross between a *lacticolor* male and a *grossulariata* female and, since *lacticolor* is a sex-linked recessive, such a cross should produce *grossulariata* males and *lacticolor* females. In this case there were 24 *lacticolor* females, no males, and a *lacticolor* gynandromorph, predominantly male. The female parent belonged to a strain, which produced many all female families. A female of this line would have either a single *X* carrying the *grossulariata* gene or no *X* at all, and the egg must have received two *X*-chromosomes, both with the *lacticolor* gene, from the sperm, non-disjunction having occurred. Thus the male parts of the gynandromorph were *XX*, and by elimination of one of the *X*-chromosomes the female parts, *X*, were produced.

The second gynandromorph was male *grossulariata* anteriorly and female *grossulariata* posteriorly. It arose from a cross between a male *lacticolor* and a female *grossulariata*. Since both the *X*-chromosomes of the male parent carried the *lacticolor* gene, it cannot have arisen from a binucleate egg, because in that case the female part would have been *lacticolor*. If, however, the insect started as a male with an *X* from each parent; and that derived from the male parent was eliminated, the male parts would be heterozygous for *lacticolor* and would show the *grossulariata* characters, and the female parts would have only one *X*, carrying the *grossulariata* gene, derived from the mother.

I know no other lepidopterous gynandromorphs, of which the origin by elimination of an *X*-chromosome can be proved, but it will be seen that in both these cases the presence of a sex-linked recessive character in one of the parents was necessary to prove that they arose in this way. *Lacticolor* is the only sex-linked form in a lepidopteron which has been bred extensively, and *Abraxas grossulariata* is a species, of which very few gynandromorphs are known. It is probable that a certain number of other gynandromorphs have originated in this way, but unless elimination of an *X*-chromosome is commoner in other species than in *grossulariata* they must be few.

(5) Theoretically non-disjunction may cause the production of gynandromorphs in more than one way.

Primary equational non-disjunction may give rise to an *XX* egg, and if this is fertilized by an *X*-bearing sperm, an *XXX* individual results. If somatic reduction then occurs at the first or at a very early cell division, with separation of the two maternal *X*-chromosomes and inclusion of the paternal *X* in one or other of the cells, the cell with *X* would give rise to male parts and that with *XX* to female parts. Morgan describes four gynandromorphs of *Drosophila*, in which the male parts contained an *X* derived from the mother. He suggests that they originated in this way, but admits that they might have arisen from binucleate eggs. An individual constituted *XXX* may also arise from an *X* egg fertilized by a sperm provided with two *X*-chromosomes due to non-disjunction.

An individual might start as a female, *XX*, and by somatic non-disjunction at a cell division, one of the resulting cells might be *X* and the other *XXX* in constitution. The *XXX* part would probably fail to develop, unless one *X* were caught

at the mid-plate, but, if this happened, a gynandromorph would be produced with male cells containing X and female cells containing XX .

An individual might start as a male, XY , and if somatic non-disjunction occurred later with the formation of XXY cells, these would produce female parts, which would form a large or small part of the insect according to the stage of development at which the non-disjunction had taken place. The earlier-formed XY cells would form the male part.

These remarks apply to *Drosophila*, in which the male is heterogametic but the phenomenon could occur equally well in Lepidoptera, though the parts with one X would be female and those with two would be male.

There is no actual proof that lepidopterous gynandromorphs arise in this way, but some are known which are female except for one male antenna or a small streak of male scales in one wing. For example there are two *Euchloe cardamines*, with a small streak of orange scales in one forewing, the rest of the insect being female. I have one with only about 50 male scales. Gynandromorphs like this cannot arise from binucleate eggs, nor is elimination of an X -chromosome a satisfactory explanation, since it is the small fraction of the insect, which has two X -chromosomes. Non-disjunction is the most likely cause, the insect starting as a female and the small patch of male tissue, XXY , being produced by somatic non-disjunction occurring at a very late stage of development.

(6) There is evidence both direct and indirect that lepidopterous gynandromorphs frequently arise from binucleate eggs, both nuclei uniting with a different spermatozoon, and a review of this has been published by Cockayne (1935). Many gynandromorphs are known, in which there is segregation of sex and of a well-defined somatic character, which is autosomal and not sex-linked. In some cases more than one such insect has appeared in a single brood or such a gynandromorph has appeared in the same brood as one, in which sex alone is segregated. In some cases the one somatic character is dominant or epistatic to the other.

In various species of *Colias* and in *Argynnis paphia* (Rhopalocera) the female parts of gynandromorph may show a sex-limited coloration, while the male parts are typical. The sex-limited colour and pattern is determined, at least in part, by a dominant autosomal gene, but even when males are homozygous for it they still retain the normal coloration, and in my opinion a second gene in the Y -chromosome is necessary for its production. If this is correct these gynandromorphs cannot arise by elimination of an X , and they probably originate from binucleate eggs.

In the case of *Argynnis paphia* gynandromorphs were produced in several generations amongst the descendants of a single female, and in many of them the female parts showed the sex-limited colour of the *valesina* form. Goldschmidt & Fischer (1927) believe that the inherited peculiarity was the liability to have eggs with two nuclei.

Goldschmidt & Fischer obtained a large number of gynandromorphs of *Lymantria dispar* in five successive generations, but since no cytological investigations were carried out and the strain showed no variation, proof that they originated from binucleate ova is lacking. A gynandromorph of *L. dispar*, which almost certainly

arose in this way has, however, been recorded by Goldschmidt (1923). It was bred from a mosaic larva, homozygous for the recessive dark colour on the right side and heterozygous for it on the left side, and its ancestry was known. Proof that binucleate ova occur in this species increases the probability, already strong, that the hereditary gynandromorphism was due to this cause.

Newman had at one time a strain of *Amorpha populi*, which produced a small number of gynandromorphs in each generation, and a few of them showed segregation of autosomal somatic characters. In one instance a female of this strain was crossed with a male *Smerinthus ocellatus* and two hybrid gynandromorphs, with one side male and the other female, were bred.

The most convincing proof is afforded by the silk-moth, *Bombyx mori*. A strain was obtained, from which many hundreds of mosaic larva were bred. In the mosaics there was segregation of an autosomal dominant and an autosomal recessive character. Some mosaics produced normal males or females, but others produced gynandromorphs. Cytological examination showed that all the ova of some females were binucleate, and it was proved that the production of binucleate eggs was determined by an autosomal gene. These results have been confirmed by Katsuki (1935), who brought an additional autosomal recessive character into his stock, winglessness in both sexes of the imago.

In the case of *Abraxas* Doncaster showed that both nuclei were egg nuclei and that each had extruded a first and second polocyte, but in the case of *Bombyx mori* Goldschmidt & Katsuki (1928) showed that one nucleus was an egg nucleus and the other a polocyte nucleus. Since no cytological examination has been made in other species, it is not known which is the commoner type of binucleate ovum.

(7) Shock. Poulton (1926), on the strength of information received from van Someren, expressed the view in a Presidential Address, that gynandromorphs of *Papilio dardanus* had been produced by mechanical shock applied to the pupa just as it was casting its larval skin. Nine were bred, four in a single family in 1925, and the other five in five different families between 1923 and 1925. Various sex-limited forms were represented in the female parts, some were very largely male, some were very largely female, and two were almost exactly halved. No explanation is given of how the gynandromorphism was brought about, but the case differs from any of those already mentioned in that half an insect is supposed to have changed its sex at a late stage of development. It seems wiser to reserve judgment, until these experiments have been repeated and the results confirmed, than to attempt to give any explanation.

VI. PARTHENOGENESIS

(1) Facultative

There are many records of facultative parthenogenesis, and a list was compiled by Tutt (1899), in which many of the following species are given. Sphingidae: *Manduca atropos*, *Sphinx ligustri*, *Mimas tiliae*, *Amorpha populi*, *Smerinthus ocellatus*. Saturniidae: *Saturnia pyri*, *S. pavonia*, *Telea polyphemus*. Lasiocampidae: *Lasio-*

campa quercus, *L. trifolii*, *Cosmotriche potatoria*, *Eutricha quercifolia*, *Macrothylacia rubi*, *Dendrolimus pini*. Arctiidae: *Arctia caia*, *A. villica*, *A. casta*, *Spilosoma mendica*. Lymantridae: *Lymantria dispar*, *Porthesia similis*, *Orgyia antiqua*, *O. gonostigma*, *Laelia coenosa*, *Psilura monacha*. Noctuidae: *Diloba caeruleocephala*, *Anarta myrtilli*. Geometridae: *Phigalia pedaria*, *Hibernia defoliaria*, *Ectropis* hybrids. Pyralidae: *Galleria mellonella*. Tineidae: *Phthorimaea operculella*, *Solenobia triquetrella*, *S. pineti*.

From this list it will be seen that it is much commoner in some families than in others, and in the case of some species there are several independent records of its occurrence. In the case of every species both males and females were produced, when the brood was of reasonable size. Meixner (1817) (quoted by Meisenheimer and others) bred one male and two females from a virgin *Lasiocampa quercus*, Kipp bred both sexes from a virgin *Amorpha populi*, Picard bred both sexes from a virgin *Phthorimaea operculella*, Garbowski bred both sexes from a virgin *Porthesia similis* and Hartmann (1912) had a race of *Bombyx mori*, in which parthenogenesis occurred regularly and he obtained equal numbers of male and female offspring. Jourdan (1861) found that with a univoltine race of this species only one out of every 3000 eggs hatched but, with a multivoltine race, one out of 17 eggs hatched, and it has been shown by others that the more broods a race produces in a year the more likely is parthenogenesis to occur in it.

Seiler found that facultative parthenogenesis occurred occasionally in *Solenobia triquetrella* and *S. pineti* and male and female offspring were bred. Weijenberg (1870) bred 13 males and 14 females from 60 virgin females of *Lymantria dispar*, and Goldschmidt (1917) after many failures had a virgin female of this species, which laid 200 eggs and from these 12 males and 7 females were bred.

Harrison & Peacock (1926b) had 8 virgin females of the genus *Ectropis*, which laid fertile eggs. From a hybrid *bistortata* ♂ × *crepuscularia* ab. *delamerensis* (melanic) ♀ they bred 3 moths, 2 males and 1 female. One male was grey, one was blacker than the mother, and the female was a low grade melanic. From a hybrid *bistortata* ♂ × *crepuscularia* ♀ they obtained 5 males and 5 females, which showed segregation of wing colour and pattern. A hybrid black *bistortata* ♂ × *crepuscularia* ♀ produced 2 black moths, a male and a female, and, since black is recessive, segregation must have occurred in this case also.

Cytological evidence is scanty, but such as it is it shows that normal maturation of the egg takes place with the formation of two polar bodies. Henking (1892) studied the reduction divisions in *Bombyx mori* and found them normal, but his eggs did not hatch. Platner (1888) also found that normal reduction took place in *Lymantria dispar*, and this was confirmed by Goldschmidt, who found that the oogonia were diploid. Seiler found that in *Solenobia*, when parthenogenesis was facultative, two polar bodies were formed and the eggs were haploid.

In all these cases of facultative parthenogenesis, in which moths were bred, there is similar evidence of segregation of sex, and in some there is also evidence that segregation of colour takes place, and such cytological evidence as exists shows that normal reduction divisions occur. It is, however, difficult to understand how

the diploid condition is regained. The ova must be of two kinds, one with an X , and the other with a Y -chromosome. Goldschmidt (1917) suggests that there is a division of the chromosomes without a cleavage division of the cell, so that one kind of egg gives a moth constituted XX and the other a moth YY , the former being a male and the latter, if viable, a female. Seiler accepts this explanation and claims to have proved that there is no X -chromosome in the parthenogenetic female of *Solenobia triquetella*, which is tetraploid, and also states that in *Talaeporia tubulosa* exceptional females occur with only 58 chromosomes possessing neither an X nor a Y . If this is so there seems to be no reason why moths with two Y and no X -chromosomes should not be viable.

Goldschmidt's explanation, division of chromosomes without cell cleavage, accounts for both sex and colour segregation in pure species and in hybrids. Harrison & Peacock (1926 *b*), however, reject it for their parthenogenetic hybrids of *Ectropis*, saying that YY individuals are unlikely to be females and are probably not viable, and discuss other possibilities. They say that with doubling other abnormalities may occur in hybrids and bring into being creatures constituted XX and XY or with recognized modifications of such constitution, whereby males and females would appear in the same broods, but admit that this does not account for cases of parthenogenesis in pure species, in which such mitotic irregularities have been proved not to occur. They consider the possibility of conjugation of the egg nucleus with that of the first polar body, but reject it because all the moths would be female and no segregation of colour could take place.

They say that there is a further possibility in the way of a chromosome explanation, and this is that actions set up and maintained by the cytoplasm may neutralize and overpower from the very beginning the forces set in motion by the chromosome complements. They conclude that any explanation of the occurrence of the two sexes in parthenogenetic broods on a basis involving the sex-chromosomes demands too many supernumerary theories to bolster it up, and think that the sex-chromosomes alone afford a hopelessly inadequate mechanism for securing the sex ratios observed in such cultures. They point out that in *Ectropis* no case of parthenogenesis has occurred in a female of pure species, in spite of many attempts to obtain it, and think that hybridization in some way sets the stage for it, or more probably originates it. They believe that the greater vigour conferred by hybridity induces segmentation to proceed, though even then it often stops and the young larvae perish.

This may be quite true, but it does not explain the numerous instances, in which segmentation has proceeded in pure species, and in any case it is in no way incompatible with the explanation that reduction divisions take place, and that eggs, some with an X and some with a Y , are produced, and that the diploid state is regained by division of the chromosomes without cell cleavage, giving males with 2 X and two sets of autosomes and females with 2 Y and two sets of autosomes.

There is, however, an insuperable objection to this explanation, if the following observation is correct. Carlier (1838) bred three parthenogenetic generations of *Lymantria dispar*, in the second of which both males and females appeared and in

the third only males. It seems impossible that males could be present amongst the offspring of females with no *X*-chromosome. The record, which is an old one, may be incorrect, and, until it is confirmed, there appears to be no adequate reason for rejecting the explanation given by Goldschmidt.

(2) *Obligatory*

Obligatory parthenogenesis occurs both in Psychidae and Tineidae. In some species males are unknown and in others there are both bisexual and parthenogenetic races. *Solenobia triquetrella* has a bisexual race and a much more widely distributed parthenogenetic one. The female of the latter is larger and Seiler (1923) has found that it is tetraploid. In the eggs of both races the maturation divisions take place, but in the parthenogenetic race only the first polar body is formed. Typical tetrads of the haploid number 30 are produced and first divide equationally to form 30 dyads, which again divide equationally in the second mitosis. The 30 dyads thus produced separate at each pole to form single chromosomes, the diploid number, and this number is maintained in the first two cleavages, but later the nuclei fuse with one another, so that the insect becomes tetraploid with 120 chromosomes in each cell.

S. pineti also has a bisexual race and a parthenogenetic race named *lichenella*, but their distribution is different, for in North Germany there is equality between the sexes, while in the South females are in great excess. The early stages are like those of *triquetrella*, but the tetraploid number is differently produced. The dyads separate in the anaphase of the heterotypic division to form single chromosomes, which are then doubled in number to 120 in the later anaphase of the same spindle, and this is regarded by Seiler as an abortive second maturation division, comparable with the formation and reunion of the second polocyte in *Artemia*. In both cases the constitution of the mature ovum is like that of the female, which produces it, and doubling of all the chromosomes does not alter the balance between autosomes and sex chromosomes, so that all the offspring should be, and actually are, females.

Obligatory parthenogenesis also occurs in *Orgyia dubia* Tausch. (Lymantridae). According to Rangnow (1912) the species is bisexual in most places, but in an area in South Russia the larvae are abnormally large and produce very large females, all of which are parthenogenetic. Males in this region are unknown. The state of affairs is very like that in *S. triquetrella*, but the cytology has not been studied.

Pictet (1924) says that in Switzerland *Orgyia antiqua* produces two kinds of female, a small one, which must be fertilized and gives both male and female offspring, and a large one with different habits, which is parthenogenetic. Both kinds of female are said to occur in the same brood, and in one case the sexes were equal in number, but there were about twice as many parthenogenetic females as ordinary ones. The data, on which these statements are based, are scanty and confirmation is required.

VII. SUMMARY

1. There are no hormones, which influence the development of the male or female secondary sexual characters, circulating in the blood, but it is probable that the corpus allatum secretes a hormone, identical in both sexes, causing the glandulae accessoriae in the male to complete their development and in the female causing the ova to mature.

2. The female sex is heterogametic, and has an *X*- and a *Y*-chromosome. Sex is determined by the correct balance between factors for maleness in the *X*-chromosome and for femaleness in the autosomes and in the cytoplasm. Their valencies differ in different species and in different races of the same species. If the valency of the *X*-chromosome greatly exceeds that of the cytoplasm, complete sex reversal may occur and an insect constituted *XY* may be indistinguishable from a normal male. When the valencies are more nearly equal, male intersexes may be produced, and these may be due to relative weakness of the cytoplasm or of the autosomes. The converse may also occur and females by sex-reversal or female intersexes may be produced. Examples of sex reversal and intersexuality occur in crosses between different races of *Lymantria dispar* and in many primary hybrids.

Intersexes are also produced by crossing a male of the bisexual race of *Solenobia triquetrella* and a parthenogenetic female of the same species. The cells in different parts of such intersexes may be haploid, diploid, or polyploid.

Intersexes occur in many secondary hybrids and possibly in *L. dispar*, and the condition is due to lack of balance between the *X*-chromosome and the autosomes. A varying number of the latter fail to conjugate and the insects approximate to the triploid state. There will be less than three but more than two sets of autosomes to three *X*- or to two *X*- and one *Y*-chromosome, giving a ratio of *X*-chromosomes to autosomes approaching 1 : 1.5, about midway between that of a male and a female. The ratio may vary in different parts of the same insect, and this may account for the coarse mosaic of male and female parts.

Intersexes are found in several species of Lycaenidae. They are restricted to certain colonies, in which there is an excess of females, and their gonads and other sexual organs are female. The cause is unknown.

Families consisting of females only or containing a large excess of females are found in various species, and three causes for this are known. In *Abraxas grossulariata* it is due to a gene, which causes the passage of the *X*-chromosome to the second polar body during the maturation of all or nearly all the ova. In *Talaeporia* it is due to non-disjunction, and in *Lymantria dispar* to a dominant lethal gene, which kills all or nearly all the males. In *Acraea* and *Hypolimnas* the cause is unknown.

3. Several sex-linked recessive characters and at least one sex-linked dominant character are known in Lepidoptera, but in comparison with autosomal characters they are rare.

Sex-limited characters occur in the females of various species, and appear to be determined by a dominant autosomal gene acting in conjunction with a gene in the *Y*-chromosome or to a gene in the *Y*-chromosome acting alone.

In *Pygaera* a sex-limited inherited cancer occurs and kills every male larva. It is transmitted by all the females to all their sons. Federley thinks that it is due to a recessive gene in the *Y*-chromosome, inhibited by the presence of an *X*-chromosome, and the action of the gene is to stimulate mitosis. He believes that the tumour is derived from the triploid polocyte. This in the female is *XXY*, and though the gene is present in the *Y* it is inhibited by the two *X*-chromosomes. In the male the polocyte is *XY* and the one *X* fails to inhibit the action of the gene in the two *Y*-chromosomes. The gene, being in the *Y*, is transmitted by every female.

4. Gynandromorphism.

(a) Boveri's hypothesis. A delay in the passage of the spermatozoon after entering the egg, so that the egg has begun to divide before the sperm nucleus reaches it and only one-half is fertilized.

(b) Only one polar body is extruded; a second maturation division takes place, and of the two ootids so formed one is fertilized and gives rise to female parts, while the other, unfertilized, gives rise to male parts. Whiting & Whiting have shown that this occurs in Hymenoptera.

(c) Morgan's first hypothesis. More than one spermatozoon enters the egg, and while one fertilizes it and forms female parts, the male parts are formed by the development of one or more of the others.

(d) Morgan's second hypothesis. An *X*-chromosome is eliminated at the first division of the fertilized egg, or at some subsequent cell division.

(e) By non-disjunction an *XXX* individual is produced, and at the first division of the fertilized egg or at some subsequent division one of these becomes separated and passes to one cell and the other two pass to the other cell. In Lepidoptera male parts would arise from the cell with two *X*-chromosomes and female parts from the cell with only one.

(f) From a binucleate ovum, each nucleus fertilized by a different spermatozoon. The two nuclei may be egg nuclei as in *Abraxas* or one may be an egg nucleus and the other a polar nucleus as in *Bombyx mori*.

(g) Mechanical shock to the pupa as it is casting its larval skin.

In Lepidoptera most gynandromorphs probably arise from binucleate eggs of the one or other kind. The elimination of an *X*-chromosome has been proved to produce gynandromorphism in *Abraxas*, but can only be proved when a sex-linked character is involved and few of these are known. One example of a mosaic arising in the way suggested by Morgan in his first hypothesis is known, so that it is possible that some gynandromorphs are produced in this way. Somatic non-disjunction is the most likely explanation of those Lepidopterous gynandromorphs, of which only a small portion is male. These would start as females (*XY*) and the male parts would be *XXY*. The production of gynandromorphs by shock requires confirmation.

5. Facultative parthenogenesis has occurred in many species of Lepidoptera belonging to different families. Both sexes are present in equal numbers in the offspring and their cells are diploid. It is probable that division of chromosomes takes place without cell cleavage and that males are *XX* and females *YY* in constitution.

Obligatory parthenogenesis is common in Psychidae and Tineidae and occurs occasionally in Lymantridae. The parthenogenetic females are tetraploid, but the tetraploid condition is produced in two different ways at least.

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ADDENDUM

While this article was going through the press, I received a personal communication from Prof. J. W. Heslop Harrison giving information about the new triploid intersexes, which he has obtained in his recent work on hybrid *Bistoninae*. Though not yet published, he has kindly given me permission to summarize them here.

(*P. pomonaria* ♂ × *L. hirtaria* ♀) ♂ × *L. hirtaria* ♀: 51 males, 35 intersexes.

The intersexes varied very much, showing a gradation from those which were almost wholly female in appearance and genitalia to those in which male and female characters were equally represented.

(*L. hirtaria* ♂ × *P. pomonaria* ♀) ♂ × *L. hirtaria* ♀: 32 males, 17 intersexes.

P. pomonaria ♂ × (*L. hirtaria* ♂ × *P. pomonaria* ♀) ♀: 10 intersexes and no males.

(*L. hirtaria* ♂ × *P. isabellae* ♀) ♂ × *L. hirtaria* ♀: 9 males, 3 intersexes, 2 dead intersexual pupae.

(*P. isabellae* ♂ × *L. hirtaria* ♀) ♂ × *L. hirtaria* ♀: 14 males, 3 intersexes.

(*L. hirtaria* ♂ × *P. lapponaria* ♀) ♂ × *L. hirtaria* ♀: 5 males, 2 intersexes.

{(*P. pomonaria* ♂ × *P. isabellae* ♀) ♂ × *L. hirtaria* ♀} ♂ × *L. hirtaria* ♀: 3 males, 2 intersexes.

I overlooked Harrison's account (*Genetica*, 1933, **15**, 115) of hybridizing.

(*Oporinia nebulata* ♂ × *O. autumnata* ♀) ♂ × *O. nebulata* ♀, which gave a brood consisting of 2 males and 4 intersexes.

The hybrid *Valeria olejaspidina*, Völker. (*V. oleagina*, F. ♂ × *V. jaspidea*, Vill. ♀) produces all males. Rorich (*Ent. Z.* 1938, **51**, 385) says that in different years more than 58 males and no females have been bred. No eggs of the reciprocal hybrid have been obtained, though copulation has taken place. It is probable that the valency of the X-chromosome of *oleagina* is much greater than that of *jaspidea*.

SEXUAL SELECTION AMONG FISHES

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I. INTRODUCTION

MANY fish exhibit a striking sexual dimorphism. Nevertheless most students of the group have failed to find any evidence that sexual selection has played a part in the genesis of these differences (Haempel, 1913; Kyle, 1926; Rauther, 1927; Hildebrand, 1930). Aside from the original views of Darwin (1871) and Wallace (1891) the supplementary suggestions of Moffat (1903), Guenther (1909) and Lebedinsky (1932) have gained little favour. In fact these later writers have made little or no reference to fishes. Very recently typical sexual selection in the Darwinian sense of female choice has been demonstrated in one of the well-known aquarium fishes, *Hemichromis bimaculatus* (Noble & Curtis, 1935). In recent years other aquarium fishes have been intensively studied, and the question may well be asked: If sexual selection is a principle of general application, why have these other students not secured some evidence of it? The answer is that sexual adornment may have several functions, and it is often very difficult to determine its particular function in any one group.

II. SEXUAL SELECTION IN THE JEWEL FISH, SUNFISH, AND FIGHTING FISH

In the experiments of Noble & Curtis¹ female jewel fish were given the opportunity of laying their eggs on one of three pots, each placed close to one side of an aquarium. On the other side of each pot, in a separate aquarium, a male or a female of the same species was placed. Experienced females always laid their eggs opposite males although the relative position of the fish was frequently changed. When one of the males had the erythrophores of its ventral surface expanded with yohimbine the females laid opposite him in 80 per cent of the cases. Injected males exhibit no courtship movements, and hence the females were responding primarily to colour. Further proof of this contention was found by placing two males, one injected, in separate aquaria at either end of a tank containing a female ready to breed. The position of the two males was frequently changed. In a series of tests with different females it was found that they remained on the average twice as long in front of the yohimbine-injected male. In each case both males were of the same size and same degree of sexual readiness.

From these experiments it was concluded that a female jewel fish ready to breed will select the brighter of two or more males. There is considerable indirect evidence to support this view. The male jewel fish, like many highly dimorphic species, selects a nest area which he defends against rivals. At this time his ventral surface becomes red. As fish of either sex approach his territory he drives them off if he is not fully ready to breed. A gravid female jewel fish under these circumstances tends to hold her ground, or at least frequently to return, with the result that she is often killed, while less ripe females and males escape. Since the territory selected by the male may be merely an upturned flower pot like many others in the same tank, it is clear that the response of the female is to the male and not to the nesting area.

It was thought at first that this tendency of a gravid female to hold her ground when attacked by a territory-guarding male served as the sole basis of sex recognition. Support for this view was secured from experiments with the sunfish, *Eupomotis gibbosus*,^{*} in the field. Nest-guarding males meet all comers with a display. The gill covers are raised, exposing their red "eyes" to good advantage, while dorsal and pectorals are erected. A rival male greeted in this manner either returns the display in kind or quickly escapes, a gravid female displays little if any and moves into the nest. If a male sunfish is killed and moved slowly on a wire towards a nest-guarding male, he will be received with all the gentle movements a male directs towards a gravid female (Noble, 1934). The marked difference in colour between the sexes is of no significance to him if the dead fish is moved without gesturing.

On the redd or nest, a gravid female circles, nudging the male as she moves. The male, thus stimulated, circles in the same manner. The eggs are laid while the female

¹ This investigation was supported by a grant from the Committee for Research in Problems of Sex, National Research Council.

bends over about 45° from the vertical and ejects her eggs at intervals towards the vent of the male. These same circling movements of the male may be induced by stroking him with a light stick while he hovers over the empty nest.

Some aquarium fishes with nest-building males seem to have the same simple form of sex recognition. The well-known fighting fish, *Betta splendens*, may be taken as an example, especially as its courtship has been recently analysed by Lissmann (1932) and by Lorenz (1935). If ripe females are placed in tanks with nest-guarding males, it will be seen that they seek the males and do not wait to be driven to the nests by the males. If a "bubble nest" of a male is moved, he will often not notice the loss but continue to guard the same part of the aquarium. When ripe females are tested in such a tank, it will be found that they seek the male and not his nest. There is, therefore, some evidence supporting the views of Köhler (1907) that, in the fighting fish, the conspicuous colours of the male serve to make him visible to the female at a distance.

Male sexual adornment serves another function, that of emphasizing gestures of dominance and thus hastening receptivity or sexual response in the female. If two breeding female *Bettas* are placed together in a tank, they will fight in very much the same manner as breeding female jewel fish. Further, the one that has been in the tank longer, especially if she has started to breed will dominate, that is, drive the other female to cover. If a male with which the female has started to breed is partly paralysed the female will usually attack him. There is, however, a difference between male and female behaviour. A nest-guarding male, if rubbed with an anaesthetized male on a fine wire, will encircle the quieted male exactly as he would a female, even though the anaesthetized male's drooping fins make him look very unlike a female. But a female *Betta*, even if dominant over several other females, cannot be induced to encircle an anaesthetized male or female by the same manipulations. The same stimulations call forth different responses in the two sexes.

A male *Betta*, like a male sunfish or jewel fish, determines if a female is ready to breed by testing her response to his attack. If adult female *Bettas* in various stages of the sex cycle are placed in a series of aquaria adjacent to one containing a male, it will be found that the latter does not show greater interest in the gravid female. If, however, a pair of breeding *Bettas* are separated by glass for a day or more and then placed together, the male will swim to the female, turn about and swim back to the nest in a peculiar undulating manner characteristic of a spawning male. Again, if two pairs have begun to breed and females are exchanged, breeding does not harmoniously continue, the males swing back to their challenge with the result that courtship must begin all over again. In brief, while it appears that the male *Betta* was merely dominating the female at the beginning of courtship, actually there is an adjustment taking place during courtship, a bond is formed with the result that breeding between the members of this pair can continue better than between any member of the pair and another fish.

Although this behaviour of the fighting fish may seem to show a surprising attention to detail, it does not compare with that of the jewel fish. In this form after the female's intention to stay in the territory has been tested with a few threats,

both sexes practise head standing which, to borrow an expression from the ornithological literature, might be called "symbolic" nest building. There is also mutual digging or pot cleaning which may degenerate into mere gesturing, but which results in the pair forming such a bond between them that they will ward off even sexually ripe individuals of the opposite sex. Close observation of courting jewel fish disclosed that it was the female and not the male who initiated the ceremonies. Further, castrated males unlike spayed females will go through a typical courtship with normal members of the opposite sex (Noble & Kumpf, 1936). Since both sexes become highly coloured during the courtship, it might be assumed that these colours enhanced the "symbolic" movements and were mutually stimulating. But the bright colours of both sexes have two additional functions; first, warning enemies which might destroy eggs or young, and secondly, to help the young recognize their parents. If young jewel fish are reared by mothers of other species having black ventral surfaces, they respond to black discs waved in the water and not to red ones (Noble & Curtis, in MS.).

Lorenz (1935) has compared the courtship of the jewel fish with that of certain birds. He assumes that two jewel fish placed in a tank will immediately display to one another. As a matter of fact this invariably happens only if the fish are in breeding condition and of the same sex. Then the jewel fish, like fighting fish, attempt to intimidate one another. A territory-guarding male jewel fish can identify the sex of an introduced jewel fish before it gestures, for he attacks the male but not the female. Since he will invariably fail in this identification if the introduced fish is anaesthetized, he is apparently discriminating on the basis of differential movements not visible to the human eye. In the jewel fish, therefore, display serves to identify sexual ripeness and not sex. In the fighting fish which display more readily and at a wide variety of moving objects (Lissmann, 1932), this distinction is less clear. The fighting fish, unlike the jewel fish, is a non-schooling species. Since sexually ripe females dominate less ripe females, the display of that sex tends to keep the area surrounding the first free of rivals.

The chief point of agreement between the courtship of sunfish, fighting fish and jewel fish is that the sexually ripe female seeks and actually comes in contact with the male. His intimidatory display which he gives to the newcomer aids the recognition of gravidity. In the first two species there may be no recognition of sex aside from gravidity. The chief difference between the courtships of the three species is that there is little if any bond formed between the pairing sunfish, only a temporary one in the fighting fish but, a strong one in the jewel fish. With the increase in the strength of the bond there is increase in the amount and variety of the ceremony. Under certain circumstances a female jewel fish may even select a territory and stimulate a male by courtship movements. Since the males develop new cycles more quickly than females, this habit is the rare exception rather than the rule. As in birds, the more complex ceremonies partake of some of the movements of nest building.

III. SIGNIFICANCE OF SEXUAL ADORNMENT IN THE GUPPY

It must be emphasized, however, that the occurrence of marked sexual dimorphism in fish is no proof that sexual selection has been at work. The guppy, *Lebistes reticulatus*, one of the best-known aquarium fishes, will illustrate. If numbers of individuals have lived together for long periods in a large tank, it will be readily noted that the males are pursuing only females. There is, therefore, contrary to a recently published opinion (Breder & Coates, 1935), a very definite sex recognition under these conditions. If, however, individuals are reared since birth in separate containers, the males will be found to attempt mating as readily with a male as with a female (Noble & Curtis, 1935). If single individuals are reared from birth with other species (*Mollienisia sphenops*), they will not school with this species, but with their own kind when given an opportunity. The inherited schooling response of guppies is visual, for blinded fish fail to school although they can find food and live for long periods in a healthy condition. In spite of this inherited tendency to keep with their own kind, male guppies reared alone will attempt to mate with a great variety of small fish introduced into their tank. In this attempt there is first a display, the male's body being bent in an arc while the fins are spread; then the fish approaching from the rear attempts to bite the vent region of the object of his attention and immediately swings his gonopodium forward to make contact with the same region.

Sex recognition in the guppy is a learned response. Male guppies, when attacked in the above manner by another, turn and bite at the offender. The latter learn to avoid males, and the male's colour pattern serves as a warning to keep away. A male which is consistently avoiding typical males will attempt to mate with an unspotted variety placed in the tank. Female guppies painted with orange and black spots are avoided by such individuals, while the same females spotted with pale green paint are sexually attractive. The presence of a gonopodium or spot of bright paint in the vent region of the receptive fish will sometimes delay the reactions of an experienced male, but spots of bright colour on the sides prevent these reactions entirely.

The response is visual, for it is made to other individuals in adjacent tanks. Further, male guppies with olfactory bulbs transected, distinguish sex and mate normally. An experienced guppy displays only to females. A dead or completely anaesthetized female calls forth no response. There must be some movement in guppies, just as in jewel fish, to induce a reaction in the male.

If sex recognition is a learned response as the above tests, frequently repeated, indicate, the display of the male would seem to be entirely superfluous. But females reared to adulthood in isolation resist the males less than gravid females do. Dulzetto (1930) has shown that, in *Gambusia holbrooki*, another species with very sexually active males, there are periods when the female is far more receptive than at other times. If a guppy is blinded and placed with a female, he is unable to mate with her. After several days of this inattention on his part she will take the initiative and make gentle advances towards him. Although this response may be a vestige

of the female approach found in territorial fish, it is so completely masked in the normal male by his sexual aggressiveness that it probably never aids normal mating. The display, another characteristic of territorial fish, seems to have no other function than to warn the female of the impending attack. Males so approached avoid the encounter or fight back, but they do not display. With the development of viviparity in the guppy and the genesis of an intromittent organ, territory sense has been lost as well as all courtship manoeuvres which would form a bond between the sexes. Nevertheless display retains its original function of a threat. The sexual colours remain permanent in adult males, and their chief function is to prevent other males from mating with their own sex. This deviation of the original function of sexual adornment seems chiefly a consequence of the development of the copulatory organ, which has made possible a rapid insemination of the female before she has had the chance to respond to the male's display.

That the conditions in the guppy are specialized and not primitive is shown by the behaviour of cyprinodontes lacking the gonopodium. In such forms as *Panchax cameronensis* (Zindler, 1914) and *Fundulopanchax coeruleus* (Meinken, 1930), the female actively seeks the males. Many of these primitive cyprinodontes, such as *Cyprinodon variegatus* (Bigelow & Welsh, 1925), are known to hold territories and in correlation with this habit the males, unlike guppies, are as large or larger than the females. In *Fundulus heteroclitus*, another territory fish, Newman (1907) observed: "When a male wishes to challenge he approaches rather cautiously, body trembling with excitement and all fins extended to the utmost, presenting as formidable an aspect as possible. The male thus challenged adopts a similar attitude and rushes at his foe with alacrity." Here display has the same function as in the sunfish.

It has been suggested that the bright colours of the Poeciliidae may make the males conspicuous to their enemies and help to divert their attention from the gravid females (Milewski, 1920). But Henn (1916) found that the sexes of *Lebistes reticulatus* were present in the ratio of 1 male to 1.2 females under natural conditions. Since at birth the sex ratio is 1 male to 2 females (Breder & Coates, 1932), mortality is slightly greater among the females in nature. Sumner (1934, 1935) has shown that the colours of *Gambusia* are protective against both bird and fish enemies; apparently the bright colours of the male guppy are of no disadvantage to it in the struggle for existence.

IV. SEXUAL SELECTION IN THE BITTERLING

The bright colours of many viviparous poeciliids may play no significant role in sexual selection, but an examination of the social relations of other dimorphic fish where sexual selection has been denied will reveal other factors to be considered. The recent conclusion of Wunder (1934) represents a case in point. The European bitterling, *Rhodeus amarus*, has the peculiar habit of laying its eggs in the gills of fresh-water mussels. The female at the time of oviposition develops a long laying tube which has recently been exploited as a "pregnancy test". The male follows

the female to the incurrent siphon and pours forth his milt into the mussel's respiratory stream. At the time of laying the fins of the male redden, his under surfaces become orange-yellow and his upper surfaces darken. Although he drives rivals from suitable mussels the female is not stimulated by these rushes. Left alone with a ripe male her tube does not lengthen, while alone with a mussel she will spawn (Wunder, 1933). Experiments with empty shells and with shells equipped with currents of water proved that both the sight of the shell and the feeling of the current were stimulating to both sexes. Wunder tested males with females having tubes of different lengths and came to the conclusion that the male selects the female with the longer tube. It cannot be argued that such females, being more sexually advanced, were more forward, hence received more attention. A long-tubed female placed in a tank of short-tubed ones attempts to hide in this strange territory, but is nevertheless sought out by the male in preference to the others. The tube holds a special interest for the male. Ordinarily the tube is flimsy and floats behind the fish like a suspended worm. The moment an egg enters the tube it becomes rigid and remains so until the egg is ejected. Noll (1877) noted that whenever a female distended this laying tube, her mate would become very excited and chase rival males with energy. Through training the tube would hold special significance for the male bitterling. Just as the male guppy has learned to avoid one type of visual cue the male bitterling has learned to be attracted by another.

Although the breeding colours of the male bitterling seem to serve primarily as intimidating devices, the question remains, might they not attract the female from afar? Here there are no experimental data to support the probability.

V. SEXUAL SELECTION IN THE STICKLEBACK

Fortunately much more is known about the European three-spined stickleback, *Gasterosteus aculeatus*, whose scarlet nuptial dress has been often discussed. The males precede the females to the breeding grounds and start the construction of their nests. They mark off clearly defined territories which they guard with vigour. The females come of their own accord and follow the male to the nest even when he is completely separated from them by a glass partition (Wunder, 1930). Only by hard biting can a male prevent a very gravid female from following him (Leiner, 1930). That the bright red dress of the male is not essential for this march to the nest was proved by Leiner (1930), who arranged his fish in a double tank, the outer containing a fluid filter which destroyed the red tone. It may be noted, however, that there is considerable ceremony attached to this simple act of attracting the female. The male first of all smears additional slime from his kidneys over the entrance of the nest. Since this act is always followed by strenuous zigzag "dancing", Leiner (1930) considers it stimulating to the male and not to the female. He believes that the male is immediately recognized by this energetic swimming motion. The female finally places her head on the male's back and is led to the nest entrance. There he pokes the entrance, turns over on his side exposing his brilliant under surfaces and permits the female to enter. Once she is inside he pushes her tail with

his belly, and Leiner (1930) has shown that, without this pushing, egg laying will not occur. The female, moreover, has a definite idea what a nest should be, for she will fail to deposit in partly built nests.

Females recognize sex before entering the nest, for they fight only among themselves and not with the pale males which have still no territory. As in *Betta*, the most sexually advanced female dominates and drives the others from the immediate field (Wunder, 1934). That sticklebacks can succeed without a colour guide is no more remarkable than the successful breeding of jewel fish in very poor light which sometimes occurs. A jewel fish without movement is of no appeal to either sex. The female stickleback responds to a certain movement of the male, as Leiner states, which movement is made more conspicuous by colour. Blinded sticklebacks with nests can readily find their way home, but are unable to engage in any sex activity (Leiner, 1929). Vision seems to be the chief sensory modality in sex recognition and sex stimulation. The visual impressions must, however, follow in a certain order. The sight of fresh eggs in a guarding male's nest will not induce him to impregnate them unless he has gone through more or less of the ceremony. Although this ceremony seems complex it is usually run through in a few minutes and the female does very little to stimulate the male. It is perhaps for the reason that no real bond is formed between the pairs, and the male may soon mate with another female.

In the related ten-spined stickleback, *Gasterosteus pungitius*,¹ the male has a black instead of a red nuptial dress and his courtship differs. The male backs away from the female and circles about before showing her the nest entrance. She never rides on his back and he never pokes her in the manner of the three-spined species, but touches with his snout. Leiner (1931) showed that the courtships of these two species were sufficiently different to completely disrupt any attempt to cross them. A striking contrast is to be found in the sunfishes, whose much simpler courtship permits the crossing of species and even genera. The point of complete agreement between the three-spined and ten-spined stickleback was that females of both species seek out the male and stand their ground in spite of his vicious onslaught. In both sticklebacks, therefore, male aggression serves to identify female ripeness. Wunder (1934), however, believes that the male three-spined species, given the choice of several females, selects the one with the greater girth. He did not apparently test females injected with salt solution. Such bloated jewel fish are not attractive to nest-guarding male jewel fish.

Proof that movement is more important than form in the courtship of the stickleback has been recently presented by Ter Pelkwijk & Tinbergen (1936). A dead female moved in the manner of a male brings forth a violent attack. Dead fish of other species, when moved slowly towards the nest with body bent in the strained attitude a gravid female stickleback assumes, will be accepted as gravid females and led to the nest. Colour pattern or form has no significance to the male stickleback provided the newcomer responds to his challenge in the manner of a gravid female.

¹ Throughout the text the scientific names employed by the authors have been quoted although many, such as this, have been relegated to synonymy by taxonomists.

Nevertheless, the male is not as completely devoid of colour discrimination as a nest-guarding sunfish. Ter Pelkwijk & Tinbergen found that a dead female adorned with the nuptial red of the breeding male is immediately attacked. Nuptial colours, therefore, help males to quickly identify rivals whether or not the latter gesture. Presumably this is a learned response, for other groups of fish, to be discussed below, make considerable use of visual cues learned after sex maturity.

Darwin (1871), in his exposition of his theory of sexual selection, pointed to the polygamous habit of the stickleback as especially favourable to the genesis of sexual adornment. Recent observations have shown that three or four females will come to a single male in the course of three days (Craig-Bennett, 1931). In many other groups of fishes having males which guard territories, two or more females have been observed to lay in the nest or area protected by a single male. This is true of such diverse types as *Cottus meridionalis* (Smith, 1922), *Cantharus lineatus* (Kent, 1883), *Badis badis* (Schreitmüller, 1915), *Gobius niger* (Riedel, 1913), *Pimephales promelas* (Markus, 1934) and *Pseudomugil signifer* (Baker, 1933). The males of these and of all other polygamous forms are, with very few exceptions, more conspicuously coloured than the female.

VI. SEX RATIO AND SEXUAL SELECTION

Nuptial adornment may have several functions. As Fisher (1930) has pointed out, there is a distinct selective advantage accruing to those individuals mating earliest. Sexual differences such as those of the guppy, which facilitate rapid sex recognition, are of value in reducing the period elapsing between sexual maturity and successful mating. Where the badge of maleness also defines territory, it is of advantage to the species in spreading the breeding group over a large area. If, however, as frequently happens, the number of suitable situations is limited, then the same warning dress becomes of advantage to the individual in that it prevents the breeding of weaker males and less-discerning females. Wunder (1930) has shown how a male stickleback drives all other males from a comparatively wide area. These other males fail to develop nuptial colours and to build nests. In the case of the jewel fish, rarely will more than one pair breed at one time in a tank of 75-100 gallons. The reason for this is that the breeding male and later female are so active in their defence of territory that they keep all other sexually mature fish in a state of agitation, which prevents them from claiming any other suitable spot in the tank for egg laying.

Darwin (1871) conceived sexual selection as functioning only at times that males were more abundant than females. The sex ratio of the species, however, may be very different from the relative abundance of males and females on the breeding ground. The sexes of the white suckers, for example, are about equally numerous; Reighard (1920) found that on the breeding shallows the males were much more numerous. Again, while Clark (1925) found that in the grunion, *Leuresthes tenuis*, the females predominated out of the breeding season, Barnhart (1918) found that on the breeding beaches the males predominated 2 to 1. The wild form of the goldfish, *Carassius auratus*, has a sex ratio of 12.9 males to 100 females under

natural conditions (Sasaki, 1926), and yet two or three males frequently chase one female during the breeding season (Tozawa, 1923).

The salmon is another sexually dimorphic fish which shows a preponderance of males on the spawning beds. Nevertheless, the sex ratio at the time of hatching is approximately 1 : 1 (Gilbert, 1923). The top minnow, *Gambusia holbrooki*, is another species which may start with a sex ratio of 1 : 1 at hatching, and yet exhibit a great preponderance of adult females in the field (Geiser, 1921; Hildebrand, 1927). The males of this species have been shown to be less able than the female to survive unfavourable environments. The high mortality among the males has presumably reduced the competition among them. Sexual dimorphism is much less marked than in the guppy, although males are frequently more or less melanistic. The breeding behaviour of *Gambusia* as described by Seal (1911) seems essentially like that of *Lebistes*. Dulzetto (1930) has shown that the fecundated female repulses the male, sometimes killing him, while the unfecundated female offers no resistance. The female attitude is one of indifference and never one of active partner-seeking such as is found in the stickleback and jewel fish.

Many factors may influence the sex ratio of a species at birth. In *Gambusia* there may be great fluctuation from the 1 : 1 average not correlated with water temperature or season (Dulzetto, 1935). In a cichlid, closely related to the jewel fish, Thumm (1908) has shown that old females mated with young males gave a progeny predominately masculine. In one brood of 800 young, not 50 were females. On the other hand, the same male, bred later with a young female, gave about 400 young, of which 300 were females. A moderate delay in the fertilization of the eggs of other fish, such as the trout (Huxley, 1923), will give a preponderance of females, while a considerable delay will cause a preponderance of males. Since sexual selection is augmented by increasing the competition between males, it follows that the amount of selection might vary greatly in any one species from year to year.

In spite of the extreme difficulty of securing by sex-ratio studies any quantitative data on the actual amount of sexual selection in a particular species, an examination of the fish on the spawning grounds has disclosed certain types of behaviour in which sexual selection may operate when the sex ratio on the breeding grounds permits. The remainder of this review will be devoted to a consideration of the better-known cases.

VII. BEHAVIOUR PATTERNS OF SPAWNING SALMONIDAE

Darwin (1871) described and figured the distorted jaws of the breeding salmon which he observes are of use "when one male charges another with wonderful violence". By way of contrast he might have pointed out that such structures are absent in Salmonidae which breed in schools. Pearl organs, horny epidermal organs which increase the friction between two fish, may be developed on one or both sexes, or there may be slight differences in size, but the colour and form of the two sexes remain essentially the same. In these species, such as the lake herring, *Argyrosomus (Leucichthys) artedi*, the males may precede the females to the shallows

where they breed. Several males may follow a female as she descends to the bottom to deposit her eggs. But there is no fighting or even dashing about in excitement (Cahn, 1927). Nor is there any restricted territory, because each female scatters her eggs over a considerable area.

In salmon and trout with their sexual dimorphism there is both territory and rivalry. It has been known since at least 1833 (Fraser) that the female of the European salmon constructs her redd unaided and the males are attracted to her as she works. Further, there are bitter contests between those males which are attracted to the redds. In striking a rival "the jaws are always clenched like a man's fist" (Seeley, 1886). Where salmon have been watched closely on the breeding grounds, no evidence of a female picking a particular partner has been observed. On the other hand, Belding (1934) has noted: "Within a period of 5 hours three different males have been observed to occupy the position of favored lover, the larger cruising males driving off their smaller rivals and usurping their position." Although the female does not go far from the redd, she may join with a large male which has taken up a position near her in chasing away the smaller males and females. Hence the female is not entirely indifferent to the competitive struggles of her suitors. Display so far as known is chiefly for the purpose of intimidation. In the rainbow trout, *Salmo gairdnerii irideus*, the behaviour is very similar to the Atlantic salmon. Greeley (1934) has shown that the smaller males give way quickly to the larger ones, while fish of equal size display before fighting. In spite of this aggressiveness, and rainbow trout sometimes fight until one male is killed, their breeding differs from that of the salmon in that a smaller, complementary male assists in the actual fertilization, by pressing against the side of the female opposite to that selected by the dominant male. Both males open their mouths during the act and the current of water holds them firmly to the redd. Under these circumstances, pearl organs found in so many other stream-breeding fish are unnecessary. The male brook trout, *Salvelinus fontinalis*, however, succeeds in a similar habitat without complementary male or pearl organs by curving his body in such a way that the female is pressed against the bottom of the redd. As long ago as 1884 Rich noted the male brook trout come first to the breeding beds, and while the female alone constructs the redd it might be assumed that the bright colours attracted the female to suitable beds. But Rich also noted: "You take the female from the bed and the male will leave; but you take a male away and the female will still remain, and before long another male will be chosen and supply the place."

Hazzard (1932) has observed one breeding female brook trout which had three successive attendants during the course of two hours. The male brook trout has a larger head, more hooked lower jaw and more brilliant coloration. The conspicuous white margin of the pectorals and ventrals are not displayed before the female. In selecting a mate the male approaches a female on her redd and stations himself just downstream of her. Courtship consists merely in an advance in position with accompanying nudging (Hazzard, 1932; Greeley, 1932). The female accompanied by a male will sometimes keep the upstream portion of the redd free of intruders. Most of the driving, however, is done by males. Hazzard (1932) notes: "Twice

during the three seasons' observations the female was seen to join the male in chasing intruders. Usually she remains indifferent to the competition going on among the males."

The male's duty of guardian of the redd continues until the eggs are covered. Then both sexes depart to participate in other mating acts. Greeley (1932) found that, in the case of several marked females, the second redd was constructed just upstream of the first redds. This suggests that it is the female who picks out the breeding area, and that she is not attracted to a particular male guarding a favourable site. In brief, all the field work on trout and salmon indicate that the female selects the territory, and she attracts males by nesting activity and males struggle among themselves for a position near her side. The display aids males in intimidating rivals. Hence sex colours in these species, like that in the guppy, helps sex identification and intimidation. They do not attract, like the bright colours of the jewel fish.

VIII. SEXUAL DIMORPHISM AND BEHAVIOUR IN STREAM FISH

In the brooks of northern United States there are fish of several families which exhibit a marked sexual dimorphism. Some of these fish guard nesting areas, and their courtship is almost identical to that of the sunfish, but in others the territorial relationships are less clear. Bright colours in the males of the latter species may merely aid sex identification, as in the guppy. It is interesting to enquire how this change of behaviour patterns may have occurred.

One of the earliest forms to be intensively studied was the rainbow darter, *Etheostoma coeruleum*. The adult males of this species are brightly tinted with orange, and they endeavour to maintain holdings in the stream bed favourable for egg laying. "Between the males colour displays are frequent. If two brilliant males are rivals and of the same size they pose side by side with their first dorsals elevated. If one of the two fishes is a small male he does not pose, but after elevating his first dorsal flees." Reeves (1907) found that the brilliant males were successful in pairing in over 60 per cent of the cases. The females, "although followed in their course by small males, often go directly from the holding of one large male to that of another. They thus appear to consciously neglect or repel the smaller and more insistent males and to give preference to the larger ones". Reeves adds, however, that this may not be a true selection of more attractive males as compared with the less attractive, but merely a selection of suitable spawning places which happen to be maintained by a liveried guard.

Male rainbow darters display to rival males and sometimes vibrate their heads in exactly the same way as they perform before a gravid female. The latter remains passive, and in this failure to erect the dorsal fin presumably discloses her sexual ripeness. In the closely related log perch, *Percina caprodes*, the male is less brilliant than the rainbow darter and ranges over the entire spawning ground instead of defending a small area against rival males. Nevertheless the mechanism of gravidity recognition is apparently the same as that of the rainbow darter. Reighard (1913) found that: "Young males in full color were often pursued by other males, and

were apparently distinguished from them only by their failure to stop and behave like females. By the experimental substitution of a male for a female it was shown that if such a male were moved rapidly and then stopped on the bottom it was treated by other males as a female."

Among the Cyprinidae there are many territorial fish, and while these are usually brightly coloured in the male sex, the activities and social relations of the various forms differ from species to species. Male northern dace, *Margariscus margarita nachtribi*, maintain holdings only some 8 in. across (Langlois, 1929) and they construct no nest. The common shiner, *Notropis cornutus*, also makes no nest but selects certain stone piles for laying (Hankinson, 1932). In the black-nosed dace, *Rhinichthys atronasus*, both sexes may sometimes cover the eggs with pebbles (Traver, 1929) but not always. In the horned dace, *Semotilus atromaculatus*, the male constructs a long ridge for the reception of the eggs. The males of all four species are equipped with pearl organs which serve primarily for holding the female during egg laying. When well developed on the head they doubtlessly assist their owners in battles with rival males, and they may aid in nest building (Reighard, 1903a). When a male approaches the holding of another he is either attacked or "escorted away". The latter "ceremony" is exactly like that in a jewel fish, and Reighard (1910) describes it as "deferred combat". It apparently arises from the fact that both fish gesture, and neither being willing to give in, both dart away parallel to one another while displaying to an extreme. In the case of the horned dace, Reighard noted that a female may come many times to a waiting male before she remains over the nest long enough to be seized by him. In this coyness she resembles the female fighting fish closely.

In the red-bellied dace, *Chrosomus erythrogaster*, each side of the male's abdomen is striped with brilliant scarlet during the breeding season, and yet there is a total absence of combat amongst the males (Smith, 1908). During laying a male flanks the female on either side while their bodies vibrate rapidly. A group of males may occupy an especially favourable laying site. Presumably their combined liveries would advertise to gravid females the position of such an area. Male stone rollers, *Camptostoma anomalum*, also breed in schools, and their brilliant orange and black dorsal and anal fins make them conspicuous. A school of males will clean a community laying area together, but Smith (1935) observed that even while working there was continuous poking and shouldering among the workers. When a female arrived there would be a mad scramble among the males crowding to her side. One or more males may mate with the female at one time (Hankinson, 1919). It thus appears that in certain cyprinids the schooling habit dominates at all seasons. Individual territorial rights have been lost. But the males still remain brilliantly coloured, for it is the females which seek the areas selected by them.

Turning to another group of fishes, the suckers, which also frequent shallow streams to breed, the problem becomes more complicated in that there are several group breeders which fail to show a sexual dimorphism other than the pearl organs. Male red-sided suckers, *Catostomus catostomus*, display bright red sides like males of many of the brook cyprinids. They never fight, but two or more pair with a

female at one time. In the red horse, *Moxostoma aureolum*, and hogsucker, *Catostomus nigricans*, the sexes are alike. The females do not mingle with the males on the rapids until ready to lay. The male challenges the new arrival with erected dorsal and protruding jaws. He also vibrates his head rapidly from side to side. Exactly the same challenge is given to another male on the spawning ground (Reighard, 1920). The female in failing to respond the same way apparently reveals her gravidity. As in the jewel fish, sex recognition can be accomplished without these gross movements. At least Reighard noted that a second male approaching a pair in contact will turn to place himself on the unencumbered side. In the suckers differences in movement rather than in colour patterns may serve to identify sex. The suckers are equipped to detect water vibrations including sounds (von Frisch, 1936),¹ while their eyes are small. In correlation with their poor territory sense, the females move on from one rapid to another and their eggs are fertilized by many males.

In many different groups of fish, whether breeding in swift or in still water, the males develop pearl organs. It is remarkable that in the silver smelt, *Hypomesus pretiosus*, a species which breeds with two males to every female, ridges have developed on either side of the male's backbone and these function as holding organs (Thompson *et al.* 1936). It might be assumed that holding organs would be of value to all breeding fish, but they appear chiefly in species in which the males struggle with one another to gain a favourable position in contact with the spawning female, or in forms where single pairs assume peculiar attitudes while mating in swift water or at least at high speed.

IX. SEXUAL DIMORPHISM IN SCHOOL BREEDERS AND IN TERRITORY FISH

There are many cyprinids which remain in schools during the breeding season and exhibit little or no sex rivalry. The goldfish, *Carassius auratus*, is a good example. During the breeding season both sexes become slightly more brilliant, and two or more males equipped with pearl organs trail the gravid female. Although they keep their snouts close to the female's vent, it does not follow that odour enters into the mating phenomenon. A gravid goldfish when decapitated fails to evoke a response in the male. Berndt (1925) found that, in certain strains which had been reared separately when placed together in a common container, the males selected females of their own strain in preference to those of others. It may well be that these different varieties had distinctive movements which the males had become accustomed to rather than to their different colour patterns. At least further evidence is required to prove that the males were responding to the different colours.

Fish that swim in schools may make their gravidity known without recourse to the gross gesturing seen in territory fish. In the goldfish it is the female which first shows her sexual readiness by vibratory movements (Berndt, 1925). In the common zebra fish of the tropical aquarium, *Brachydanio rerio*, it is well known that

¹ *Biological Reviews.*

the female first drives the male (Schreitmüller, 1912; Baake, 1928*a*). After various nudges the male turns and drives the female in a very lively chase about the tank, during which the eggs are laid. Although there is no sexual difference other than girth, the fishes are not confused as to sex even during these rapid dashes. In the related genus, *Barbus*, the eggs are also scattered by the parents, but here the males of at least many of the Asiatic species are more brilliantly coloured than the females. The appearance of the sexual dimorphism is again correlated with the development of the territory habit. In such species as *Barbus conchoni* and *B. oligolepis*, aquarists have frequently seen the males select particular areas among the water weed which they defend against rival males (Baake, 1930, 1933*a*; Engelhardt, 1927). In brief, where tropical cyprinids maintain their schools throughout the breeding season, no sexual differences of colour usually appear, but the retirement of breeding males to territories is almost invariably correlated with a change of colour or at least an increase of brilliancy in their nuptial dress.

The same correlation is found in other families of fish. The Characidae include many tropical species commonly reared by fanciers. Most species of the family show little sexual difference of colour. In such familiar forms as *Hemigrammus caudovittatus*, *H. ocellifer*, *Creagrutus beni* and *Hyphessobrycon flammeus* the female is said to first drive the male in the manner of *Danio* (Hildebrand, 1930; Baake, 1933*b*; Thomas, 1934; Schreitmüller, 1925). In these forms there is again little rivalry among the males. Although typical pearl organs do not appear, some species develop hooks on the anal fins of the males which have the same function, namely, holding the females (Schoenfeld, 1935). In this family, as in the cyprinids where species develop clear-cut territory habits, the males become much more highly coloured than the females. In *Copeina guttata*, for example, the male digs a pit in the sand in the manner of cichlids, but the eggs are caught and fertilized in the bent anal fin as in many other characins (Stolzenhain, 1926). After laying the male drives off his mate and guards the nest. The male's equipment of carmine spots presumably has the same double function of the breeding dress of male jewel fish, they attract the attention of the sexually ripe female and they make the male's threat to egg robbers more conspicuous.

Where fish, because of their large size or pugnacity, are capable of guarding a territory efficiently, there may be territory holding without the development of an intimidation dress in the male. The black bass, *Micropterus dolomieu*, for example, has very similar habits to the sunfish, and yet fails to exhibit any conspicuous colours. The laying female differs from the male in being conspicuously blotched, but this spotting may not appear until after she has entered the nest (Reighard, 1906). As in the sunfish, the male builds and guards the nest, but unlike that conspicuous species the male never elevates his gill covers to challenge the female when she arrives (Reighard, 1906). In the absence of display, sex recognition is presumably accomplished by the identification of movement as in the jewel fish, but the gravid female has a decidedly greater girth than the male. In some instances the male may find a female in deep water and bring her to the nest (Lydell, 1902). At least the male black bass ranges much farther afield than the breeding sunfish, and a large

one may even appropriate the female from the nest of a smaller male. In general, however, the "females when ready to spawn come from the deeper water, one after another, and skirt along the shores searching for a mate. When the female comes within range of the vision of the waiting male, he rushes out to meet her" (Beeman, 1924). Whether it is because of the ability of the male to identify sex without display, or because of his greater aggressiveness in seeking a mate, or merely because of the usually more exposed nest, the male black bass has been able to succeed in the business of reproduction without developing the bright colours of the male sunfish.

It may be noted, however, that there are various territory fish whose nuptial colours have been described as actually protective and not at all conspicuous in the habitats they select. The lumpfish, *Cyclopterus lumpus*, is such a case (Cox & Anderson, 1922), and the bowfin, *Amia calva*, another (Reighard, 1903*b*). Males of the latter species construct their nests among rushes at no great distance from shore. The breeding male differs from the female in having the lower surfaces orange-yellow, fading to cream-white below, and these bright tones would presumably advertise his presence to the female in the same way as the bright ventral colours of the male sunfish presumably function. Reighard (1903*b*) has studied the approach of females to the nest and the details of egg laying, "In no case was there seen any posing of the male with fins spread in front of the female, such as occurs in some teleosts, nor was there any other evidence that the male used his colors as a sexual excitant. Since spawning takes place chiefly at night, such use of the colors of the male is not to be expected."

Proof that males can detect the presence of females at a distance was apparently afforded by an experiment of Reighard (1903*b*) who staked out a slat crate containing a number of females. The crate drew males guarding eggless nests from a distance of 30 m. One male excavated a nest immediately beneath the crate and two others in the vicinity.

X. ODOUR IN SEX RECOGNITION

In some fish there is evidence that odour may enter into sex recognition. Eggert (1931) believes that a cutaneous gland found in the vent region of the male *Blennius pavo* functions in attracting the female to the cracks or holes where the male secretes himself during the breeding season. As in the sunfish, several females may deposit their spawn in the nest occupied by a single male. Since the male ordinarily does not leave the hole until a female arrives, any mechanism which attracts should have some selective value.

On the other hand, many fish which have been described as utilizing olfactory cues in identifying the opposite sex may actually employ very different methods. Among the lampreys, for example, it is said by Roule (1931) that the male attracts the female from afar by secretions from its body. In the brook lamprey, *Lampetra wilderi*, Reighard (1903*c*), however, has found no evidence that odour enters into sex recognition. Both sexes engage in building the community nests in stony

brooks. When a male seizes another male with his suckorial mouth the latter releases his grip on a rock, and as the pair drift downstream the two fish separate. Females containing eggs, if seized by males while attached, retain their hold and begin at once to "shake". The male reacts to this movement by throwing his tail in a loop about her and rapidly vibrating all of the body behind the branchial region. The breeding female *L. wilderi* is distinguished from the male by an oedematous second dorsal fin. This apparently serves as a support for the tail of the male which is thrown accurately in the notch formed by the first and second dorsals. Only the females are equipped with anal fins, and these may serve to dislodge sand or other debris between the stones of the nest. In brief, while the structural differences between the sexes of *L. wilderi* may facilitate the grip of the male and the attachment of the eggs, the methods of sex recognition seem to be so simple that sex differences in colour such as occur in the sunfish would be of no value.

Reighard (1903c) marked breeding individuals of *L. wilderi*, and found that there was no relation between a particular fish and a particular nest. One male may mate with several females, and several males with the same female. Coventry (1922) has also witnessed one male of the sea lamprey, *Petromyzon marinus*, mate with two females in the same nest. In the lake lamprey, *P. marinus unicolor*, however, the males often precede the females to the breeding ground. They are much less social than the brook lamprey, and there is some rivalry among them, at least after the females arrive (Surface, 1898). There is, on the other hand, no rivalry among the females, even when ovipositing. Early in the season the males predominate on the breeding grounds, towards the middle of the season the sexes are about equal in number, but towards the end there may be as many as five females in a nest with one male. The pugnacity of the males has led to the formation of incipient territories, or even partial polygamy. The species is far less social than *Lampetra wilderi*, breeding individuals tending to remain scattered instead of aggregating. Hence here at the base of the vertebrate series, it is the presence of male pugnacity and the lack of aggregation drive which has marked out discrete breeding territories in the case of one species of the group. No sexual dichromatism has evolved, and apparently, in correlation with the poor visual apparatus of the lamprey. The struggle for territory is, however, not keen and this in turn may have had an effect. The lake lamprey, it may be pointed out, is not entirely devoid of defence mechanisms. The brook lamprey, due to the degeneration of the teeth, is incapable of inflicting a wound. In the better-equipped lake lamprey, Surface (1898) has seen a male battle with another for the lordship of a spawning bed which contained a female. When the first male struck the intruder the latter writhed with all evidence of pain. It seems that the lake lamprey can identify a male without gripping him. Such a capability has doubtlessly assisted the male in holding his territory.

XI. NUPTIAL COLOURS AS INTIMIDATING DEVICES

It has been frequently noted in birds that the greater the display the less the fight. The same rule seems to apply to fish. Fishes have a well-developed colour sense, and they learn differences in colour quicker than differences in shade

(Schaller, 1926). A threat of colour comes through learning to have significance for a fish. In referring to the bizarre and brightly coloured dragonet, *Callionymus lyra*, Holt (1898) remarks: "Occasionally the two males meet in full splendour. Then one lowers his colours and flies ingloriously; but I have seldom seen anything in the shape of a fight, and have never found wounds that might have been inflicted when I was not watching." The same display is given to young males or unripe females with the result that both flee. If the challenge is made to a gravid female, "acceptance is denoted by the female swimming to the side of the male, who, as a rule, instantly lowers his fins and retracts his jaws and gill covers" (Holt, 1898). The pair then press closely together as they swim upward in the act of egg laying.

A brightly coloured fish may, however, first display and, if the disturbing factor still remains, then fight. The jewel fish follows this procedure. In the family Cichlidae, to which the jewel fish belongs, most species develop conspicuous colours usually red or black during the breeding season. If both sexes develop these nuptial colours the courtship is mutual as in the jewel fish and both sexes take care of the young. Where, however, the female after a courtship essentially like that of the jewel fish takes full charge of the young and drives the male away, she alone retains a brilliant colour after egg laying. This is characteristic of the genera *Apistogramma*, *Heterogramma* and *Nannacara*. The females are often much smaller than the males, and yet their pugnacity is such that the males flee soon after fertilization (Dörschel, 1934; Stoye, 1935; Ludwig, 1935; Waldmann, 1936). Conspicuous colours or at least contrasting colour patterns appear in all cichlids which form attachments between pairs lasting beyond egg laying. In the gentle scalare (*Pterophyllum*) there appears to be less rivalry than in the jewel fish, for two females may deposit spawn together (Armbruster, 1934). There is nevertheless a definite claiming of territory by mating fish (Ritschl, 1917) and both sexes become very dark (Günther, 1916). At this time they emit a clicking sound which may help them to intimidate trespassers on their domain.

When cichlids give up all defence of territory for their young, only the male is brightly coloured. Since their courtship may be very similar to the jewel fish, the males' bright colours presumably serve to attract females and make their dominating gestures more conspicuous. This group includes the "mouth breeders" which avoid defence of a home territory by carrying their young in their mouths. In some species the female broods, in others the male, and yet it is always the male which is the most conspicuous. The mouth-brooding habit evolved within the Cichlidae and several intermediate steps are found within the genus *Geophagus*. The species which approaches the mouth breeders most closely, namely, *Geophagus jurupari*, shows very little increase in colour during the breeding season. This reduction of nuptial adornment is correlated with the fact that rivals rarely fight (Härtel, 1936).

Considering the family Cichlidae as a whole it may be said in brief that conspicuous colours are of advantage to all males in their courtship, for their behaviour is essentially like that of the jewel fish. Bright colours in the female would seem to strengthen the bond between mating pairs, for both sexes gesture "symbolically" in essentially the same way. Bright colours may also intimidate enemies which

would devour eggs or young. Where the pair remain mated until only after the eggs are laid the female may successfully dispense with bright colours. The nuptial colours of the Cichlidae seem to have the important function of making the movements and gestures of breeding or brooding fish more conspicuous.

Not all fish which guard their young are brightly coloured. Species which are active at night and are provided with poor eyesight would have little use for the brilliant display of the jewel fish. The common bull-head, *Ameiurus nebulosus*, is a good example. The species claims territory (Eycleshymer, 1901; Ward, 1926) and both sexes excavate the nest. The males fight among themselves (Stranahan, 1910), and this tends to spread the nests over a large area. Vision is poorly developed and, unlike the condition in cichlids, is rarely used in feeding (Schiche, 1921). The tactile organs on lips and barbels are very sensitive to mechanical stimuli (Hoagland, 1933), and adult fish in an aquarium frequently run their barbels over one another, suggesting that these organs may be used in sex discrimination instead of the eyes.

The dwarf catfishes (*Corydoras*, *Callichthys*, etc.) are obviously specialized forms, and yet they retain the barbel play of *Ameiurus* during courtship. In *Corydoras* at least there is no rivalry among the males which are smaller than the females but essentially like them in colour. Some authors such as Reitz (1910) speak of the female making her selection among her suitors. In *Corydoras paleatus*, the best-known species, the first indication of spawning is a restless swimming of the female up the sides of the tank. Only later the males are aroused and pursue the agitated female. One or more will swim close to her and bring their barbels in contact with the top of her head. As this "feeling" continues the males become more and more agitated until one of them causes the female to pause for a moment. Then, vibrating his whole body, he throws himself in front of her snout and clamps the female's barbels between his nearest pectoral fin and the side of his body. The female presses her mouth tight against the side of his body while he quivers rapidly. As the eggs are ejected they are caught in the pelvic fins of the female brought together to form a pocket and are inseminated by the male who bends his body in several sharp contractions. The female takes no sperm in her mouth, as frequently reported (Boecker, 1909; Liebig, 1924; Roehlike, 1933), for the good reason that her mouth is never brought in contact with his genital pore. The roles of touch, olfaction and taste in this strenuous mating behaviour have never been worked out, but it is clear that there is no territory, no challenging of the female and no attempt to intimidate rivals. In correlation with these facts there are no sexual differences in colour.

In other groups of fishes without territory or intimidation there may be courtship behaviour very different from that of the catfishes. Among the seahorses, for example, the male invites the female to mate by approaching her with gaping brood pouch. Weber (1924) found that a male *Hippocampus brevisrostris*, repulsed by one female, rapidly swims to another. Hence there is little bond between the sexes, the male assuming a provocative position before several females until one responds by depositing eggs in his sac. Although the male stimulates the female by a display of his person, this is not adorned with distinctive nuptial colours.

Some seahorses are said to produce sounds during the breeding season (Gill,

1905). In other groups of fish where this occurs it replaces the colour display of gaudy relatives. Hence *Ctenops vittatus*, for example, a close relative of the fighting fish, builds a bubble nest which the male protects. A passing female is challenged by the same fin gestures of *Betta* but, as if to compensate for their sombre tones, the male also growls loudly (Stampehl, 1931). Both sexes of *Betta* display and both sexes of *Ctenops* make these sounds (Beyer, 1931). Apparently the growl, like the display, serves to dominate the female, bringing her to the next stage of courtship.

XII. SIGNIFICANCE OF NUPTIAL COLOURS IN VIVIPAROUS FISH

As shown above, most nest-guarding fish employ bright colours as intimidating devices. There are, however, many species such as the guppy discussed above which are ornately coloured in the male sex and yet claim no territory. In a few of these the bright colours may actually attract the female. In *Cynolebias adloffii*, for example, a poeciliid which lays its eggs in a large number of small pits prepared by the male, Adloff (1925) describes how a female will follow and nudge a male not ready to mate. The picture is very similar to that of a gravid jewel fish attracted by the bright colours of a male which has not yet claimed territory. In most egg-laying poeciliids, the males of which fail to claim territory, there is little evidence of male attraction. Males which are ornate or conspicuously marked, such as *Haplochilus lineatus*, as described by Baake (1928b), drive the female until she finds a suitable place in the aquarium for egg laying. The males fight bitterly among themselves, and their sexual adornment is apparently a warning to rivals. Since a male may select a female of another species in preference to his own (Arnold, 1925), it would seem that learning entered into the process of sex recognition as in the case of the guppy.

The live-bearing poeciliids are often extremely bedecked in the male sex, and the question may well be asked, is this coloration only for intimidation? In the sail-bearing *Mollienisia*, the sail is erected not only for fights but also for courtship (Baake, 1932). Stolzenhain (1927) describes the courted female as lying on her side to make the contact of the gonopodium easier. In most species of this group, as in the diminutive *Heterandria formosa*, the male has been frequently described as stealing up from behind and thrusting the gonopodium forward before the female was aware of what was happening (Henzelmann, 1928; Baake, 1929). Since, as Brüning (1918) has shown for the guppy, five broods of young may result from a single fertilization, there would seem to be little need for the great sexual activity which characterizes most species of the group.

Darwin (1871) described and figured the swordtail, *Xiphophorus helleri*, and compared the adornment of this viviparous poeciliid with that of gallinaceous birds. One obvious difference between the two groups is that the viviparous fish fails to claim a breeding territory. The long, golden sword which protrudes from the ventral margin of the tail fin is assumed by Van Oordt (1925) to be more than a decoration. He describes the male as constantly trying to touch the genital papilla of the female with it, and assumes that it serves to stimulate the female erotically.

Kosswig (1936) agrees with Van Oordt in assuming the sword functions as a sexual stimulant, but he claims it is rubbed along the side of the body of the female. Since *Xiphophorus helleri* produces an excess of males (Bellamy, 1924) there would seem to be a real competition between them, and presumably the most stimulating males would pass on their effective swords to the next generation. While this would not account for the brilliant colours of the sword, it might at least explain how selection may have favoured its growth in length.

A closer examination of the problem, utilizing the same methods employed in the study of the guppy, has shown that the bright colours are actually intimidatory devices.¹ The swordtail courts in the manner of a guppy, but instead of bending its body in an arc during the display it backs up and touches the side of her body with his sword. Swordtails are more pugnacious than guppies and the males fight each other in the presence of a female. Sex recognition is purely visual, for the sex of swordtails in adjacent glass aquaria is readily recognized. As in the case of the guppy, males reared from birth in isolation fail to recognize sex but attempt to mate equally often with both sexes. Male swordtails learn rapidly when exposed to both sexes, and they do not forget as rapidly as do guppies. A male swordtail with both his sword and gonopodium removed will be mistaken for a female, but his vicious response to males which attempt to mate with him will soon result in his being left alone. A young male equipped with gonopodium but with only a sword rudiment is also mistaken for a female if his gonopodium is removed. Size may enter into sex recognition. If a sword is attached to a small female swordtail she is treated as a male by other males, but if the same sword is attached to a very large female she is courted and mated with in spite of the decoration. All this attention to detail is found only in adult males which have been reared with females. Sexually starving a male will cause him to mate with an immature male which has already developed a gonopodium. In brief, sword, gonopodium and frequently size are visual cues utilized by experienced male swordtails in identifying the male sex. As in the jewel fish a completely immobile swordtail arouses no sexual interest in another. Given some movement and sex is identified, not by differential movements as in the jewel fish, but by attention to the same gross details of form and colour the aquarist ordinarily employs to identify the fishes' sex.

Although the golden sword of *Xiphophorus helleri* may serve the same important function as the conspicuous spots of the guppy in permitting males to rapidly identify sex, it may nevertheless have other functions. While driving a female the male frequently curves his tail in a way to prevent the female's escape. This technique is especially successful in corners. The sword is gently rubbed against the body of the female during courtship, and it is vigorously thrashed towards a male during a fight. The sword is part of the tail and in both jewel fish and swordtail that organ is gently slapped towards a female and vigorously directed towards a male. Hence the sword has the double function of stimulating the female and intimidating the male. To what extent this stimulation of the female is actually effective is not known.

¹ A full account of this work as well as that on the guppy will be published elsewhere by Noble and Borne.

In the ordinary course of events the males pursue the females so vigorously that they do not have a chance to respond.

XIII. CONCLUSIONS

1. It has been shown experimentally that female jewel fish, *Hemichromis bimaculatus*, will select the most highly coloured of several possible mates. Further this selection will be made when only visual cues are available to her.

2. Although female choice has played a role in the development of sexual adornment in fish, nuptial colours may have several functions in the group.

(a) They may serve to threaten rivals or enemies.

(b) They may emphasize gestures which are essential to the formation of nuptial bonds. Some of these gestures are "display" but others are "symbolic nest building."

(c) As a result of learning, nuptial colours may serve to identify sex and to increase the efficiency of breeding behaviour.

(d) Nuptial colours may aid young fish to learn to distinguish their parents from adults of related species.

3. The pattern of breeding behaviour has an important influence on the general sexual adornment.

(a) In most territory-guarding fishes the males are conspicuously coloured and the females are conspicuous if they defend the nest or young. Exceptions occur in forms with poor vision and in some of large size.

(b) In most school breeders there is no sex dimorphism in colour, although pearl organs or other devices for holding the female may be present. Exceptions occur when the males as a group select suitable spawning areas and wait *en masse* for the females to arrive.

(c) In most viviparous species, although the female facilitates mating during certain periods, the male is usually so sexually active that the female has little opportunity of making a selection. The development of the gonopodium makes possible for the male to take the leading role in mate selection. Such males rarely alone mate with both sexes, and sex identification is a matter of learning. The male nuptial colours serve as a warning for other males to keep away.

4. The female reveals her state of gravidity:

(a) In territory fish by approaching the territory-guarding male and then passively resisting his challenge.

(b) In a few forms such as the salmon by actively engaging in nest building before the arrival of the male at the nest site.

(c) In some school breeders by failing to flee or to gesture with her fins in the manner of a male; in others by driving the male or by vibrating her body.

5. In territory-guarding fish nuptial colours aid breeding because the female stimulated by the display pass quickly into the later phases of courtship. In school breeders and non-territory fish where displays do not aid the recognition of

gravidity, long drives may occur before the female is sufficiently stimulated to proceed with egg laying.

6. The sex ratio of fish on the spawning grounds is often very different from the sex ratio at other seasons. By considering the former ratio together with the behaviour pattern of spawning the functional significance of the nuptial colours in any one species may be determined.

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(d) Nuptial colours may aid young fish to learn to distinguish their parents from adults of related species.

3. The pattern of breeding behaviour has an important influence on the genesis of sexual adornment.

(a) In most territory-guarding fishes the males are conspicuously coloured, and the females are conspicuous if they defend the nest or young. Exceptions occur in forms with poor vision and in some of large size.

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SOIL CONDITIONS AND THE ROOT-INFECTING FUNGI

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I. INTRODUCTION

THE relation of fungus plant diseases to the environment has formed the subject of a number of general reviews, amongst which may be mentioned those by L. R. Jones (1924), Butler (1925), Brooks (1928*b*) and Foister (1935). The influence of soil conditions upon the soil-borne diseases of plants has received perhaps less than its fair share of attention in such reviews, though this is to be ascribed to the omissions of investigators rather than of reviewers. Whilst the soil as an environment for the activities of its autochthonous microflora has engaged the attention of soil microbiologists for upwards of seven decades (Waksman, 1936), mycologists appear to have lagged behind in their study of the soil as a habitat for the plant pathogenic fungi. No complete review of soil conditions and the root-infecting fungi appears to have been attempted yet, if we except the treatment of this subject in textbooks by Waksman (1931) and others. Mention must certainly be made, however, of the review by L. R. Jones *et al.* (1926) of the extensive studies carried out at Wisconsin upon the relation of temperature to soil-borne diseases of plants.

The effect of soil conditions upon soil-borne diseases has attracted most attention from two aspects in particular. In the first place, soil conditions may determine

the geographical distribution of diseases. The broader limits are set by variation in soil temperature with latitude (L. R. Jones *et al.* 1926), whilst local distribution may be conditioned by soil type. In the second place, local variation in the incidence of diseases suggests the possibility of control through soil management. Control by modification of the environment is certainly more feasible with soil-borne than with air-borne diseases, since the soil environment can be considerably modified by agricultural practices, e.g. by methods of cultivation, manurial treatments and soil amendments, crop rotation, time of planting, etc.

Soil conditions may affect a soil-borne disease either by acting directly upon the fungus, or by modifying the resistance to attack of the host plant. In addition to this, variation in any one factor may cause concomitant variation of still greater importance in one or more other factors of the soil environment. Thus soil moisture and soil aeration are very closely interrelated, since the available pore space of the soil is shared between the soil water and the soil atmosphere, and any variation in one will cause an inverse variation in the other. Again, soil temperature and moisture content may operate upon a disease not only directly through their effects upon the fungus, and upon host resistance, but also indirectly by influencing the factor of microbiological antagonism to the parasite (Garrett, 1934).¹

The influence of the environment upon host resistance has been reviewed at length by L. R. Jones *et al.* (1926), Brooks (1928*b*), and Brown (1934, 1936), and it is therefore not proposed to go far into this complex subject here. The influence of the "environmental coincidence" upon host resistance has been dealt with by Jarvis (1932) from another angle; he considers that the susceptibility of a crop to disease decreases as the environment approaches the "ecological optimum" for the host.

In some cases, an unfavourable environment may merely predispose the host to disease, as in the browning root rot of cereals in Canada, the distribution of which has been correlated by Vanterpool (1935) with a high nitrogen/phosphorus ratio of the soil, although the disorder is actually caused by *Pythium arrhenomanes* and certain other species of *Pythium*. The disease can be effectively controlled by application of superphosphate. In other cases, however, some defective soil condition may be the real cause of disease, and the associated fungus infection merely an effect. Thus although the heart-rot disease of sugar-beets had been demonstrated by Gäumann (1925) to be rather closely, though not invariably, associated with infection by *Phoma betae* in the soils of Switzerland, the disorder was later proved by Brandenburg (1931-32) to be due to boron deficiency of the soil. Again, Gerretsen (1935) has claimed that bacteria are concerned in the etiology of the typical leaf-spot symptoms of the grey-speck disease of oats, the cause of which was established by Samuel & Piper (1928-29) as a deficiency of available manganese in the soil. Other unfavourable soil conditions, such as severe nitrogen deficiency, have been shown by Rayner (1934) to upset what normally appears to be a comparatively benevolent association between a mycorrhizal fungus and its host plant, so that the fungus symbiont becomes parasitic, and, in a similar manner, deficiency of the soil

¹ *Biological Reviews.*

in available boron may cause root-nodule bacteria to become destructively parasitic in their leguminous host plants (Brenchley & Thornton, 1925).

It is thus impossible to neglect the factor of host resistance in any consideration of the environment in relation to plant diseases. Due allowance for the operation of this factor will accordingly be made in section III of this review, when considering the effect of different soil conditions upon individual soil-borne diseases. For further general consideration of host resistance, reference should be made to the reviews already cited. In the present paper, attention will be devoted more particularly to the influence of the soil environment upon the ecology of the causal organisms. The soil environment of a pathogenic fungus comprises not only physical and chemical, but also biological components, in particular the host plant and the other soil micro-organisms. Most attention will be given to the biological factors in section II, whilst section III will be devoted mainly to the consideration of the physical and chemical factors of the soil environment.

II. ECOLOGY OF THE ROOT-INFECTING FUNGI

Soil conditions may operate directly upon a fungus in both the parasitic and the non-parasitic phases of its life underground. During the parasitic phase, they may affect not only the process of infection, but also the rate of external spread of the fungus over the subterranean parts of the host plant, where such external spread occurs. During the non-parasitic phase, the survival of the fungus in the absence of its host must again be greatly affected by the soil environment. It does not follow, however, that those conditions optimum for the parasitic activity of a fungus will also be those most favourable for its survival in the non-parasitic phase; indeed, the converse situation is more likely to be found.

(1) *Activity of fungi in the parasitic phase*

Comparatively little is known of the growth habit of parasitic fungi in the soil, but positive observation is beginning to replace mere speculation, and some previously held views have had to be considerably modified. At one time, it was considered that many of the root-parasitic fungi were capable of independent and even unlimited growth through the soil. Such a view derived partly, no doubt, from the fact that all these fungi could be grown readily enough on artificial media, and were thus in no sense obligate parasites. The conditions of pure culture, viz. an abundant and suitable food supply and absence of competition, can rarely be realized in the soil, however.

Certain parasitic fungi, however, are undoubtedly capable of making free and independent growth through the soil. It is significant that these belong to the class of parasites primitive and undeveloped in their parasitic relations with the host (Brown, 1936) and with a wide host range. Thus the fungus *Rhizoctonia solani* may be microscopically observed to make at least a limited hyphal growth through the soil. Certain other parasitic fungi make a growth through the soil that may be visible to the naked eye. Thus the mycelium of *Rosellinia arcuata* and *R. bunodes* is

stated by Petch (1923) to grow through the upper layers of soil in the tea plantations of Ceylon, a profuse development occurring especially in the presence of much humus. When the spreading mycelium encounters a tea bush, infection occurs, and the death of the bush may result. The white mycelium of *R. necatrix*, causing white root rot of fruit trees, has been observed by Nattrass (1927) in England to spread visibly through the soil in a similar manner. The same habit of growth has been described by Nowell (1923) for various species of *Rosellinia* occurring in the West Indies. A more highly developed type of growth through the soil is shown by *Armillaria mellea*, in which the exploration hyphae are banded together into rhizomorphs, root-like organs showing some tissue differentiation. Rhizomorphs are not confined to the root-infecting fungi, being developed also by *Merulius lacrymans*, the dry rot fungus, which is thereby enabled to spread long distances over inhospitable substrata, such as brickwork or cement (Brooks, 1928a). By means of its rhizomorphs, *Armillaria mellea* was found by Ellis (1929) to travel 22 yards through fissures in the bare rock. *A. mellea* is, however, a comparatively unspecialized parasite with a very wide host range, and is considered by Day (1929), Rayner (1930) and Reitsma (1932) to attack only under conditions which have to some extent weakened the host, although it is reported by Dade (1927) to be a virulent parasite of cacao in the Gold Coast Colony.

Evidence is accumulating, however, to show that the majority of the more specialized root-parasitic fungi are almost entirely confined in their activity to the underground organs of their host plants, and that their spread through the soil is therefore conditioned by the root contact of their hosts. This now appears to be the case for four very different plant diseases of great economic importance, viz. the root rots of rubber due to *Fomes lignosus*, *F. noxius*, and *Ganoderma pseudoferreum*, root rot of cotton due to *Phymatotrichum omnivorum*, take-all of wheat due to *Ophiobolus graminis*, and Panama disease of bananas due to *Fusarium cubense* (*F. oxysporum* f. 3). The three fungi causing root rots of rubber have been studied by Napper (1932-34) who has shown that, for all practical purposes, infection by these fungi spreads only along the roots, and from plant to plant by root contact. Although it is true that the rhizomorphs can also spread along other solid surfaces in the soil, such as boulders and dead roots, this fact is not of much practical importance, since these fungi can only use living roots as a nutritive substrate, and are unable to invade dead and decayed roots already occupied by other organisms. The rate of spread of these fungi is considerably more rapid in clean-cleared and clean-weeded plantations of young rubber than in those in which secondary jungle (belukar) is allowed to grow up. The close root network under such secondary jungle is considered to exercise a "baffling" effect upon the advance of the rhizomorphs, which are split up into a great number of tributaries; the rate of forward spread is then much reduced. These and other interesting aspects of the rubber root-disease situation in Malaya have been discussed at length by Sharples (1936).

The cotton root rot disease, caused by *Phymatotrichum omnivorum*, shows many points of similarity with the rubber root rots. The strands of this fungus seem to have a strictly limited power of independent growth through the soil. In loosely

packed natural soil in laboratory containers, a growth of 14 cm. was obtained by King *et al.* (1931) and one of 10 cm. by Taubenhaus & Ezekiel (1930a). Both sets of observers found the fungus to grow much more readily along the glass walls of the vessel than through the soil, suggesting the preference for a continuous solid surface also shown by the rubber root rot fungi. Whilst the strands of this fungus thus appeared capable of independent growth through the soil for a short distance under favourable conditions (e.g. loose packing of the soil), the field studies of Taubenhaus & Ezekiel (1930a) very convincingly demonstrated that the spread of *P. omnivorum* in a cotton field was by growth along the roots, and from plant to plant by root contact. A contrary opinion had previously been expressed by Peltier *et al.* (1926), but on very slender evidence. The rate of spread of this fungus has been observed by King (1923) and others to be much more rapid in cotton fields than in fields of alfalfa, a plant equally susceptible to the fungus. By analogy with the root rots of rubber, this might be tentatively attributed to the fact that the root network in a cotton field is of larger mesh than that in a field of alfalfa.

A similar state of affairs was revealed in the take-all disease of wheat by Padwick (1935), who showed that the causal fungus, *Ophiobolus graminis*, could apparently spread through the soil only along the roots of wheat plants.

In each of the foregoing diseases, the fungus spreads along the outside of the host root, at the same time penetrating the tissues by means of branch hyphae, which serve to supply the external hyphae with nourishment. In the Panama disease of bananas, the causal fungus, *Fusarium cubense*, spreads inside the root along the vascular system, emerging only at a later stage of the infection. Cousins & Sutherland (1930), from observations that no spread of infection occurred from diseased areas surrounded by a zone cleared of plants, concluded that this disease spread through the soil only by root contact. Indeed, the practical recognition of spread by root contact in the case of this disease may be said to date from the initiation of control by rogueing, i.e. the removal of the surrounding plants in root contact with the infected individual. The results of the application of this method of control to Panama disease are discussed at length by these authors. A somewhat similar method is employed in the control of the rubber root rots in Malaya, which is described by Sharples (1936); the infection is traced along the diseased roots, which are then either appropriately treated or destroyed, together with the original source of infection, in the course of a periodical tree-to-tree inspection of the plantation. Considerable control of the *Verticillium albo-atrum* wilt of potatoes by the "three-plant method" of rogueing, in which the wilted individual and its two neighbours in the row are pulled up, has been reported by McKay (1926).

Summarizing, it may be said that parasitic fungi may spread underground in three ways:

- (1) Freely through the soil and over the underground organs of the host plant, e.g. *Rhizoctonia solani*, *Rosellinia* spp. and *Armillaria mellea*.
- (2) Along the outside of the host organs (which are immediately or subsequently invaded by the fungus) e.g. *Fomes lignosus*, *Phymatotrichum omnivorum*, and *Ophiobolus graminis*.

(3) Along the inside only of the host organs, e.g. *Fusarium cubense* and the other wilt-producing vascular *Fusaria*.

The direct effect of soil conditions upon the fungus may be expected to diminish down the above series, in which the subterranean spread of the fungus becomes increasingly independent of the direct incidence of the soil environment, but at the same time more and more dependent upon host resistance as affected by soil conditions. Thus in the case of those fungi growing along the outside of the host organs, soil conditions may well determine whether an infection shall be slow of advance or even non-progressive, or whether it shall spread more or less rapidly in either direction. Thus with the take-all disease of wheat, Garrett (1936) has shown that in a soil favourable to *Ophiobolus graminis* the fungus may spread rapidly up and down the root system from a single focus of infection. In a soil unfavourable to the fungus, on the other hand, infection may or may not be inhibited altogether; if it does occur, it is either non-progressive or else advances only slowly. It is thus easy to understand why soil conditions should so markedly affect the prevalence of this disease. On the other hand, with fungi in which spread is confined to the inside of the host organs, soil conditions can directly operate only on the actual process of infection, exerting merely an indirect influence upon the rate of spread inside the root, through modification of host physiology.

(2) *Survival of fungi in the non-parasitic phase*

A parasitic fungus may survive in the soil apart from the living host in four ways: (i) as actively growing mycelium, (ii) as conidia or as resting spores, (iii) as sclerotia, (iv) in dead infected host tissue. Certain parasitic fungi, such as the *Rosellinia* spp. cited above, appear to be capable of living and growing upon the soil organic matter; indeed, their parasitism appears to be incidental, and they can probably persist in the soil as actively growing mycelium for an indefinite period. The majority of the soil-inhabiting parasitic fungi, however, seem to depend on the presence of host plants for their continued existence in the soil, and can only survive for a limited period in infected host tissue, or in the form of conidia, resting spores, or sclerotia. In general, it may be said that fungi which form resting spores or sclerotia at the conclusion of a phase of parasitic activity are thereby enabled to survive in the soil for periods usually much exceeding their term of viability in infected host tissue alone. The survival of the thin-walled conidia, however, is probably of short duration, though the period may not often be so brief as suggested by the experiments of Werner (1935), who failed to find conidia of *Fusarium nivium*, causing wilt of water-melons, in naturally infected soil, though the mycelium of the fungus was present in abundance. If conidia were introduced into the moist soil, they vanished within 4 days, their disappearance being attributed to their assimilation by the other soil organisms.

Resting spores, on the other hand, have been shown by various investigators to survive comparatively long periods in the soil. The variation in reported survival periods may be illustrated by a few examples from the extensive literature, viz. 2 years or more for *Aphanomyces euteiches* (Geach, 1936), 3 years for *Sclerospora*

graminicola (Hiura, 1935), and 4 years for *Plasmodiophora brassicae* (Gibbs, 1931). Reported field observations suggest that for quite a number of soil-borne diseases the period of survival may be considerably longer still, but discussion of this possibility will be deferred till later. Turning now to those investigators who have studied the effect of different soil conditions upon the longevity of resting spores, mention should be made of the observations of Dixon *et al.* (1935) on the influence of seed-bed treatment upon the overwintering of oospores of *Peronospora tabacina*, causing blue mould of tobacco, in old seed beds in North Carolina, as shown by the occurrence of primary infection foci in the spring. Soil conditions appear to influence very markedly the survival of chlamydospores of the cereal smut fungi. Thus in the experiments of Borzini (1935), the spores of *Ustilago zaeae*, the maize smut, were found to survive better on the surface of soil than when buried in the soil, and, in the latter case, better in air-dry than in moist soil. The spores of *Tilletia tritici*, the cause of bunt in wheat, were found by Hanna & Popp (1934) to survive in the soil best of all in bunted heads of wheat, next best in bunt balls (spherical aggregations of spores), and least well when the bunt balls were broken up so that the individual spores were in contact with the soil. In artificially infected soil, the percentage infection caused by the fungus was found by Hungerford (1922) to fall from 100 to 4.5 per cent after incubation of the soil for 1 month. The fungus was reported to survive the winter in the soil in Canada (Hanna & Popp, 1934), but not in Utah (Tingey, 1934). The spores of *Urocystis tritici*, causing flag smut of wheat, were found to overwinter in the soil in the U.S.A., but loss in percentage viability was very considerable (Griffiths, 1924; Tisdale *et al.* 1927). In Australia, the spores of the fungus readily survive the hot dry summer in the soil (McAlpine, 1910).

The survival of sclerotia is similarly influenced by soil conditions. Sclerotia may be defined as aggregations of resistant hyphae; such bodies are of variable size and shape, but generally show the differentiation of an external layer composed of especially resistant hyphal cells. The long survival of sclerotia of *Phymatotrichum omnivorum* in Texas is attributed by Neal & Wester (1932) to the anaerobic conditions prevailing in the subsoil of the black waxy clay lands on which cotton is especially grown. In this connexion, it is interesting to compare two experimental studies which have been made of sclerotia survival in *P. omnivorum*. Taubenhaus & Ezekiel (1936) obtained a 10–15 per cent survival in sclerotia buried in clay soil in closed containers after a period of 5 years. On the other hand, King & Eaton (1934), using a light sandy soil in open containers, found viability to be reduced to about the same figure at the end of 1 year. This lack of agreement between the results of the two sets of investigators is interesting, inasmuch as it may be put down to the differences in soil aeration and other conditions obtaining in the two experiments. McNamara *et al.* (1934) found *P. omnivorum* to survive in a pseudo-sclerotial form as "persistent strands" for at least 3 years in the field. Both King & Eaton (1934) and Taubenhaus & Ezekiel (1936) found the sclerotia of *P. omnivorum* to be singularly susceptible to dry conditions, the former reporting a survival of only 2 months, and the latter one of less than 9 days in air-dry soil. The sclerotia

of other fungi, however, seem to survive best in air-dry soil. Thus the sclerotia of *Rhizoctonia solani* were found to survive 4-5 months in moist soil, but 6-7 months in air-dry soil (Palo, 1926). The sclerotia of *Sclerotinia oryzae* remained viable for 4½ months in moist soil, but for 6½ months in air-dry soil (Park & Bertus, 1932). Sunderaraman (1928) found the sclerotia of *Rhizoctonia bataticola* to retain viability for as long as 15 months in air-dry soil.

The survival of a fungus in infected host tissue buried in the soil is a question of considerable microbiological interest. It was at one time considered that after the death of the host organ, the parasite continued to feed on the tissues until all the food material was exhausted. This may certainly be true of some fungi; thus *Fomes lignosus* is stated by Napper (1932*a*) to continue growth in an infected rubber root until this is reduced to a mere shell. With certain other fungi, however, the initial parasite is rapidly followed up by secondary organisms. The succession of organisms developing in a diseased root provides a good example of *micro-ecological succession*. The parasite proper, in virtue of its specialized character, is able to colonize a new habitat, the plant root. Once this habitat has been opened up by the parasite as the first colonizer, however, it is quickly invaded by other organisms, the general type of succession being probably related both to soil conditions and to the changes occurring with decomposition in the invaded root. Such a succession is undoubtedly analogous to that of higher plants occurring in any new subaerial habitat, such as a sea-shore sand-dune. The occurrence of such a succession of organisms in diseased roots is recorded by Hansen (1929) for the pink root disease of onions, following upon the initial invasion by *Phoma terrestris*, the true parasite. Isolations made from infected roots at different stages of decay showed that certain *Fusarium* spp. followed *Phoma terrestris* with such regularity as to suggest that they were secondary parasites rather than chance invaders. This indication was confirmed by pathogenicity tests, in which it was found that onions inoculated with a combination of *P. terrestris* and the *Fusarium* spp. suffered greater damage than those inoculated with *Phoma terrestris* alone; inoculation with the *Fusarium* spp. alone produced no effect, however, showing that these fungi were incapable of unaided parasitism on healthy onions. The rapid development of secondary parasites and saprophytes in infected root tissues explains the difficulty sometimes experienced by plant pathologists in isolating the true pathogen from roots not in the earliest stage of infection. Thus the pink root disease of onions had been originally attributed by Taubenhuis & Mally (1921) to *Fusarium mali*. Again, in the case of the *Rhizoctonia solani* disease of cereals, described by Samuel & Garrett (1932), it was observed (unpublished) that a species of *Helminthosporium*, probably identical with the *Helminthosporium* M of Henry (1924), followed *Rhizoctonia solani* with great regularity; whereas isolations made from recently infected roots would yield *R. solani*, the true parasite, isolations made subsequently sometimes gave nearly 100 per cent of the *Helminthosporium* sp. but no *Rhizoctonia solani*. Further examples are quoted by Machacek (1928), in reviewing association in plant pathogenic organisms.

Instances of similar micro-ecological successions in the saprophytic fungus

flora of the soil and other habitats are easily brought to mind. The changes occurring in the fungus flora of fresh horse-dung when incubated under a bell-jar in the laboratory will be familiar to most botanical students. The succession of organisms developing on fermenting cacao has been recently described and discussed by Knapp (1935), with especial reference to temperature. An interesting mycological investigation of the microflora developing on dried, cut slices of sugar beet has been reported by Pidoplitscha (1930), who found that the organisms developing on the slices varied characteristically both with temperature and with the moisture content of the tissues.

The survival of a fungus in infected host tissue will depend not only upon the rapidity with which it is replaced by other organisms, but also upon the rate of decomposition of the infected tissue. The rate of this process will vary according to the particular fungus parasite, the nature of the host tissue, and general soil conditions. Thus *Fomes lignosus* destroys rubber root tissues much more rapidly than does *Ganoderma pseudoferreum*, and this difference is considered by Sharples (1936) to explain the greater longevity of the latter in dead infected roots. In the second place, survival will naturally be favoured by woody or otherwise resistant substrata slow of decomposition in the soil. Thus an outbreak of the *Fomes noxius* (*F. lamaoensis*) brown root disease of tea in northern India was traced by Tunstall (1930) to a piece of infected *Mesua ferrea* wood which had lain buried in the soil for at least 14 years. In general, however, the period of survival in infected host tissues is very much shorter than this. Thus in Texas, Taubenhause & Ezekiel (1930b) reported that *Phymatotrichum omnivorum* was able to survive the winter on living infected roots but not on dead decayed ones; furthermore, healthy cotton plants were successfully inoculated with the tap roots of infected plants 2 weeks, but not 3, after the death of the diseased plant. These conclusions were supported by those of Neal & McLean (1931). The survival of *P. omnivorum* for more than 3 years on the site of infected roots was found by McNamara *et al.* (1934) to be due to the formation of "persistent strands", closely resembling sclerotia in structure and function, though unusual in their elongated form. Luthra *et al.* (1935) in India found that infected residues of the gram crop if left on the surface of the ground would carry *Aschochyta rabiei* for more than 2 years; if buried in the soil, however, the contained fungus was killed in 1 month, provided the soil was sufficiently moist. Under tropical conditions, the higher soil temperatures must promote a decomposition of infected residues considerably more rapid than that obtaining in a temperate climate (Corbet, 1935; Jensen, 1936). A study at present in progress on the survival of *Ophiobolus graminis* in infected wheat straw buried in different soils indicates that rate of disappearance of the fungus depends both upon the nature of the host tissue, and upon soil conditions. In brief, it may be said that conditions favouring general microbiological activity in the soil favour also the decomposition of infected host tissues and the disappearance of the fungus.

In this connexion, mention may be made of the important studies of Weindling (1932-34) who found (1932) that the very common soil-inhabiting fungus *Trichoderma lignorum* was able to attack and decompose the hyphae of various other

parasitic fungi in the soil. He further discovered (1934*b*) that a number of other common soil fungi possessed the same power of parasitizing other fungi, but none were so active in this respect as *T. lignorum*. In a detailed study (1934*a*) it was shown that the parasitism of *T. lignorum* was effected through the excretion of a "lethal principle", which decomposed very rapidly in alkaline media, but comparatively slowly in acid media. Close correlation was obtained between the concentration of the "lethal principle", and the vigour of parasitism by *T. lignorum* of the host hyphae. A biochemical study of this "lethal principle" has been made by Weindling & Emerson (1936). In logical application of these studies, Weindling & Fawcett (1936) were able to obtain satisfactory control of *Rhizoctonia solani* causing damping-off of citrus seedlings by adjusting the soil reaction to pH 4.0, which was optimum for parasitism of the *R. solani* hyphae by *Trichoderma lignorum*. Whilst the work of Weindling at present remains the most detailed study on the parasitism of an individual soil-inhabiting fungus, similar studies on other soil micro-organisms may show this parasitic capacity to be comparatively widespread. Thus it is stated by Waksman (1931) that, under certain conditions, organic matter is quickly broken down by the soil fungi with the formation of abundant mycelium, which at the conclusion of activity is as rapidly decomposed by the soil bacteria.

At the same time, the role of microbiological antagonism (Fawcett, 1931) in the soil, for so long neglected, is now perhaps in danger of being over-emphasized. During the parasitic phase, the activity of a pathogenic fungus is scarcely likely to be much hampered by the antagonism of the soil saprophytes except in soils to which fresh organic matter has recently been added. Thus in pot experiments with the cereal foot-rot fungi, microbiological antagonism appeared to be of importance only in those experiments in which cultures of the pathogenic fungi on cooked cereals or other such rich organic substrata had been employed (Garrett, 1934). Again, it has been possible to demonstrate in a very striking manner (Sanford & Broadfoot, 1931; Greaney & Machacek, 1935; Endô, 1935) the control of soil-borne diseases by means of specific saprophytic organisms introduced into sterilized soil, for this medium is particularly favourable for their activity, on account of the fresh food material liberated by sterilization of the soil organic matter. The growth of a parasitic fungus may be completely suppressed by that of a saprophyte on culture media (Broadfoot, 1933; Endô, 1935) or in sterilized soil (Henry, 1931), but this is scarcely surprising, since the true saprophytes will frequently grow more vigorously on such media than will the parasites (or facultative saprophytes). It thus becomes dangerous to press the analogy between laboratory and greenhouse demonstrations, on the one hand, and field conditions, on the other, too far.

(3) *Soil-inhabiting and soil-invading fungi*

If a parasite so quickly disappears from a habitat in which it has had the initial advantage of an abundant food supply and no competition, it is difficult to imagine that it can compete with the true saprophytes in the general life of the soil. Indeed, the common practice of crop rotation for the control of soil-borne diseases *ipso facto* implies the disappearance of a fungus parasite from the soil in the absence of

its host or hosts. Of especial interest in this connexion is a series of studies made by Reinking & Manns (1933-34) and by Reinking (1934-35) on the soil *Fusaria* of tropical America. As a result of extensive soil isolations, they concluded that certain *Fusarium* spp. could be classified as *true soil inhabitants*, in that they occurred in every soil investigated, and were therefore to be regarded as common soil saprophytes. Other *Fusarium* spp. were found only very locally, and always in association with past or present sites of host plants; with the death of the host plant, they gradually disappeared from the soil, and were hence designated *soil invaders*. This valuable conception of soil-inhabiting and soil-invading fungi has, indeed, done much to clarify a somewhat confused situation. How often are parasitic fungi recorded in general lists of soil fungi, made by different investigators (Waksman, 1931)? A study by Henry (1931) of the distribution of *Helminthosporium sativum*, the cause of the foot-rot disease of wheat, in a Canadian wheat soil showed this fungus to be present in only 1 per cent of the soil samples taken, but it occurred, on the other hand, in 10 per cent of the wheat stubble bases selected at random from the same field.

On the other hand, very high numbers of such a *soil invader* may be found in soils carrying a succession of severely diseased crops. Thus, from soils under continuous cropping with flax, Bolley & Manns (1932) obtained as many as 45,000 colonies of *Fusarium lini*, the flax wilt organism, per gram of soil. Glynne (1925) obtained severe warting of potatoes by *Synchytrium endobioticum* in experimental tests only when the number of resting sporangia of the organism exceeded 1000 per gram of soil. Various investigators (Naoumoff, 1928) have demonstrated the existence of a correlation between the severity of club-root infection in cruciferous crops and the number of *Plasmodiophora brassicae* spores in the soil.

(4) *Propagation and dispersal of the root-infecting fungi*

Reference has already been made to field observations which seem to indicate that a number of fungi responsible for soil-borne diseases can survive in the soil for many years in the absence of a susceptible crop. Thus Barker (1923) stated that it was frequently impossible to grow flax varieties susceptible to wilt caused by *Fusarium lini* more than once every 10-12 years on the same land, Melhus *et al.* (1926) declared that the cabbage yellows organism, *F. conglomerans*, could live for at least 11 years in the soil, Linford & Vaughan (1925) observed the occurrence of severe outbreaks of *Aphanomyces euteiches* root rot of peas 10 years after the land had carried the last pea crop, and Schaffnit (1922) reported that potatoes contracted wart disease on land which had been kept fallow and free of weeds for 10 years. It is quite possible that the thick-walled oospores of *A. euteiches* and the resting sporangia of *Synchytrium endobioticum* may be capable of surviving in the soil even for a period of 10 years. It scarcely seems likely, however, that the chlamydospores of the two *Fusarium* spp. could survive in soil for periods so long as those reported above.

The clue to such long apparent survival of fungi lacking either especially resistant resting spores or sclerotia may lie first in susceptible weeds acting as

alternate hosts, and secondly in reinfection of the land through various agencies. The importance of eradicating weed carriers of the causal organism on the fallows and amongst non-susceptible crops has been stressed by many investigators, and most recently by Taubenhaus (1936) for *Phymatotrichum omnivorum*. Infection of virgin land, and reinfection of land freed from a parasitic fungus, can occur in a diversity of ways. Reinfection may be brought about by the distribution of spores or infected material carrying the fungus, by the agency of wind, water, and animals (ranging from insects to human beings).

Taking first dispersal by water, Bewl y & Buddin (1921) have drawn attention to the contamination of glasshouse water supplies by dangerous parasites. Wardlaw (1935) considered that *Fusarium cubense* might be distributed by the flooding of rivers, and King *et al.* (1934*b*) have brought forward evidence of the spread by erosion and drainage water of *Phymatotrichum omnivorum*; Thung (1932) has studied the distribution by drainage water of *Phytophthora nicotianae* causing black shank of tobacco in Java.

Wind distribution of infected debris has been cited by Crawford (1934) for the *Fusarium annuum* wilt of pepper, and by Luthra *et al.* (1935) for the *Aschochyta rabiei* gram blight, as well as by many others. It seems quite possible that wind may play an important part in the epidemiology of a number of soil-borne diseases through dispersal of their aerial spores, but few studies have been made of this agency of distribution. Samuel & Garrett (1933) have suggested that aerial dispersal of ascospores in showery weather is responsible for the widespread occurrence of the take-all disease in South Australian wheat crops in certain seasons. These epidemics seem to occur only in seasons of good spring rainfall, such as would provide the necessary conditions for spore discharge, spore dispersal, and infection to take place. Spore dispersal is here considered to occur through the growing crop; the ascospores may be washed down by rain into actual contact with the wheat roots. Although precise data are not available, it is unlikely that such delicate aerial spores can persist for long in the soil if they fail to make contact with a host root.

Infection by air-borne spores has been considered by Bryce (1922), Petch (1928), Gadd (1936) and others to play an important part in the ecology of the root rots of tea, rubber, cacao and other tropical crops. The severe outbreaks of root rot occurring in plantations established on the site of felled tropical jungle were attributed to widespread infection of the jungle stumps by air-borne spores of the root parasites. No satisfactory experiments to substantiate this hypothesis have been performed, however, and the theory has been severely criticized by Briton-Jones (1934) and by Napper (1932*a*). The observations and experiments of Napper (1932-34) on the rubber root rots in Malaya indicate that root infection is widely distributed throughout the natural jungle, but in such a mixed population, a biological equilibrium has been established between the jungle trees and their parasites, so that the effect of the latter is scarcely apparent. After felling and burning the jungle, the young rubber trees are planted, constituting a uniform population of susceptible hosts, in which the "latent" jungle infections then develop, giving rise to scattered patches of diseased plants. The activity of the root

parasites is probably further stimulated by the change in soil conditions brought about by the felling and burning of the jungle; the effect of these operations upon general microbiological activity in Malayan jungle soils has been studied by Corbet (1935). An interesting parallel to this sequence of events is afforded by the occurrence of severe outbreaks of the take-all disease in wheat grown on land ploughed up from mixed pastures containing species of grasses susceptible to the fungus. In this case, again, the "latent" infection may be widely distributed throughout a mixed population showing no very apparent evidence of disease. Such observations as the above have done much to remove the *raison d'être* of the air-borne stump-infection hypothesis, which has thus lost its chief support. Furthermore, Napper has found that *Fomes lignosus* is especially sensitive to the antagonism of saprophytic organisms, and is unable to infect dead and partially decomposed roots already occupied by other organisms; this factor of competition must be borne in mind by those postulating the colonization by parasitic fungi of tree stumps open to invasion by numerous wound-parasites and saprophytes.

Whilst the occurrence of stump infection by air-borne spores of a root parasite is probably of much less ecological importance than formerly supposed, the observations of Gadd (1936) suggest very strongly that it does occur in the case of certain fungi, viz. *Fomes noxius* and *Ustulina zonata*, after the felling of shade trees on the tea estates of Ceylon. On the other hand, direct infection of the roots by means of such spores falling on the soil is no longer considered likely. Thus Tunstall (1930), De Jong (1933), Gadd (1936) and many others have pointed out that infection by root-disease fungi frequently fails to occur in the absence of an adequate nutritive substrate behind the infection hyphae. Inoculations with spores or mycelium from pure cultures have frequently given negative results in infection experiments with these fungi, whereas inoculations with large pieces of infected root have almost invariably been successful (Tunstall, 1930; De Jong, 1933; Taubenhaus *et al.* 1929). Gadd (1936) has suggested that this need of the infecting hyphae for a "food base" is probably the factor limiting the spread of infection by many fungi to root contact. Only those fungi which can use the soil humus as a food base, e.g. the species of *Rosellinia*, can escape this restriction. The effective parasitic range of rhizomorphs or strand hyphae from a food base may thus be much narrower than observations of visible spread through the soil would suggest. This might explain, for instance, the apparent contradiction between laboratory and field observations in the case of *Phymatotrichum omnivorum* (Taubenhaus & Ezekiel, 1930*a*), to which reference has already been made. The general relation of nutrition to the physiology of parasitism has been recently discussed by Brown (1936).

Investigations into the possibility of spore dispersal in certain other root-infecting fungi have also yielded negative or inconclusive results. All attempts to produce infection by the conidia of *P. omnivorum*, the cotton root-rot fungus, have failed, and Taubenhaus & Ezekiel (1931) conclude from their observations that there is no field evidence for spore dissemination of this fungus. The foregoing observations have been cited to show that the mere production of aerial spores by a

root-infecting fungus need not necessarily imply that such spores assist at all in its dispersal. At the same time, spore suspensions have been successfully employed to produce experimental infection in a variety of root diseases too numerous for individual mention. What is now needed, therefore, is a series of field studies on the epidemiology of some of these diseases, similar to that made by Keitt & Jones (1926) on apple scab, a foliage disease.

The dispersal of soil fungi over small areas is assisted by the movements of the soil fauna, and over wide areas by those of insects, higher animals, and the cultivator. Gleisberg (1922) found earthworms to carry the spores of *Plasmodiophora brassicae* through the soil. The relation of insects to the dispersal of plant diseases has been recently reviewed by Leach (1935). Gibbs (1931) has cited examples of the spread of *P. brassicae* over New Zealand farms by infected soil carried on implements, the feet of farm animals, and the boots of farm workers; the carting of infected roots as feed for stock also contributed to the spread of the club-root organism. The rapid extension of flag smut of wheat in New South Wales was attributed by E. S. Clayton (1925) to the feeding of farm horses on infected hay, a dangerous practice already condemned by McAlpine (1910).

In contradistinction to the long apparent survival of a fungus in the absence of its appropriate cultivated host, there is the converse situation, in which the natural dying out of a parasite has been recorded under continuous cropping with a susceptible host. Such natural dying out of infection is especially apparent in the case of *Phymatotrichum omnivorum*, and has been studied by a number of investigators, but most thoroughly by McNamara *et al.* (1931). These authors followed the behaviour of root-rot spots in Texas by mapping the distribution of plants killed by the fungus each season on land planted continuously (i.e. every spring) to cotton, which is an annual crop. In this way, it was found that the limits of such a root-rot spot expanded farther into the healthy crop every year. Inside the spot, however, infection would die out in some places and not in others, apparently at random. After a few years, such a spot might practically disappear between one crop and the next, in that only a few isolated foci of infection would remain to mark out the limits of the once more or less uniformly diseased area. Such dying out appeared to be independent both of soil type and season, and, indeed, a disappearing spot might be closely adjacent to one which was expanding. It seems probable that such natural dying out of the fungus is due not to any hypothetical auto-intoxication, already largely discounted by the experiments of King *et al.* (1931), but simply to the natural disappearance of the fungus from the soil on the death of the host plant, and the destruction of that equilibrium upon which the survival of the parasite, as well as that of the host, must depend. Indeed, the successful maintenance of such an equilibrium is generally held to be a characteristic of the more highly developed parasites (Brown, 1936). For upon the death of the host roots, the activity of the fungus is severely checked, and it may in its turn suffer decomposition by the other soil organisms. Whether an infection shall survive the period between successive crops will depend to some extent upon the length of this period; if the host be killed at an early stage by a virulent attack of the parasite, the

infection is less likely to survive than if the living host carried a mild infection until the end of the growing season. Moreover, an early death of the infected host may cut short the formation of sclerotia or other survival organs by the fungus. In this way, therefore, a progressive yearly development in the intensity of attack must almost inevitably be followed by a natural decline. Thus in the case of these cotton root-rot spots, the first plants to die are reported by McNamara & Hooton (1929) and others to be always those in the zone just outside the limits of the previous year's spot, which zone would have been occupied in the previous season by plants infected too late to show visible signs of the disease. The plants inside these, i.e. on the site of those actually killed by root rot in the preceding season, often remain healthy till late in the season. The foregoing explanation of the behaviour of these root-rot spots is substantially that suggested by Taubenhaus & Killough (1923). It received support from the experiments of Taubenhaus & Ezekiel (1930*b*), in which it was shown that *P. omnivorum* could survive the winter only on living infected roots and not on dead decayed ones. This explanation does not seem to be accepted by McNamara *et al.* (1931), in spite of the fact that their field observations are not in disagreement with it, and indeed, appear rather to offer it strong support. Taubenhaus & Ezekiel (1931) have recently again discussed the question.

An apparently similar phenomenon has been observed by Fellows (1934), who found that a patch of the take-all disease in wheat, appearing in one year, might increase in size and virulence during the second year, decline in the third, and might not reappear in the fourth year. Again Glynne (1935), in a survey of the incidence of the same disease in experimental plots planted continuously to wheat over a period of three consecutive years, found a marked decrease in percentage plants infected during the third year, but only in those plots in which infection exceeded 35 per cent in the second year. In those plots showing less than 35 per cent infection in the second year, there was a further increase in percentage infection during the third year. It may be suggested that in the more severely infected plots (infection exceeding 35 per cent in the second year), the plants were killed at an earlier stage, with detriment to the survival of the fungus over the period intervening before the sowing of the next crop.

III. COMPONENT FACTORS OF THE SOIL ENVIRONMENT IN RELATION TO SOME SOIL-BORNE FUNGUS DISEASES

Most of the published work has been done on comparatively few diseases—those of most economic importance—so that the field of knowledge is necessarily somewhat limited. For the purpose of this review, a survey has been made of the published work of the last 15 years on soil-borne fungus diseases, with the object of collecting information as to their prevalence under different soil conditions. Also included in this survey are certain diseases caused by organisms belonging not to the true fungi, but to two related groups, the Actinomycetes and the Myxomycetes; their inclusion is justified both by their widespread occurrence and economic importance, and by the attention which they have received from mycologists. The

information thus obtained has been summarized, and is presented below under the headings of the different factors of the soil environment, viz. soil moisture content, texture, organic matter content, reaction and chemical composition. The effect of soil temperature upon plant disease will not be dealt with here, since it has been reviewed at length by L. R. Jones *et al.* (1926) on the basis of work done by these authors and their collaborators at Wisconsin. In the second place, the effect of soil temperature upon plant disease is intimately connected with host resistance, a subject which cannot be adequately treated here. In this section of the review, strict economy has had to be exercised in the citation of authorities, and a selection has therefore been made, having regard to the general interest and value of the papers quoted, as well as to their immediate relevance. For further information as to the different plant diseases cited, and for concise accounts of the causal organisms, reference may be made to Brooks (1928*a*).

(1) Soil moisture content

Diseases known to be favoured by high soil moisture content are given in Table I. The first eleven of these diseases are caused by organisms in which dissemination of the disease through the soil and infection of the host plant can be

Table I. *Diseases favoured by high soil moisture content*

Fungus	Disease	Authorities
<i>Plasmodiophora brassicae</i>	Club root of crucifers	Monteith (1924), Naoumova (1933)
<i>Spongospora subterranea</i>	Powdery scab of potatoes	Blattný (1935)
<i>Synchytrium endobioticum</i>	Potato wart	Glynn (1925), Esmarch (1926)
<i>Aphanomyces euteiches</i>	Root rot of peas	F. R. Jones & Drechsler (1925), Haenseler (1926)
<i>Pythium arrhenomanes</i>	Root rot of sugar-cane	Flor (1930), Carpenter (1934)
<i>P. arrhenomanes</i>	Root rot of corn	Johann <i>et al.</i> (1928)
<i>Sclerospora graminicola</i>	Seedling blight of Italian millet	Tasugi (1935)
<i>Phytophthora cactorum</i>	Crown rot of rhubarb	Beach (1922)
<i>P. cambivora</i>	Ink disease of chestnuts	Blin (1922)
<i>P. cinnamomi</i>	Pineapple wilt	Lewcock (1935)
<i>P. parasitica</i>	Various	Bewley (1923), Petri (1929)
<i>Fusarium annuum</i>	Chilli pepper wilt	Crawford (1934)
<i>F. cubense</i>	Banana wilt (Panama disease)	Wardlaw (1935)
<i>F. lycopersici</i>	Tomato wilt	E. E. Clayton (1923), White (1926)
<i>F. orthoceras</i> var. <i>pisi</i>	Pea wilt	Starr (1932)
<i>Fusarium</i> sp.	Celery yellow	Ryker (1935)
<i>Armillaria mellea</i>	Mushroom root rot	Dade (1927), Gard (1927)
<i>Calonectria graminicola</i>	Snow mould of cereals	Eleneff (1926)
<i>Helminthosporium sativum</i>	Foot rot of cereals	McKinney (1923)
<i>Sclerotinia sclerotiorum</i>	Collar rot of lettuce	Soursac (1922)
<i>Sphaerostilbe repens</i>	Violet root rot of tea	Tunstall (1922)

brought about by the agency of free-swimming zoospores under conditions of high soil moisture content. The favourable effect of high soil moisture upon such diseases can thus be largely interpreted as acting directly upon the causal organism. The next five of the diseases listed above are so-called vascular wilts caused by species of *Fusarium*; since these fungi make extensive growth within the vascular system of the host plant, it is possible that high soil moisture in some degree favours the progress of infection by inducing a "soft" or succulent type of growth in the

host especially favourable to the parasite, as has been suggested by E. E. Clayton (1923) in the case of tomato wilt due to *F. lycopersici*; increased susceptibility was also promoted by other measures, such as heavy nitrogenous manuring, inducing "soft" growth in the tomato plant. Again, rising soil moisture content may reduce soil aeration to such an extent that root injury occurs, and the entry of certain parasitic fungi is thereby much facilitated. Thus *Armillaria mellea* and *Sphaerostilbe repens* are both considered (Day, 1929; Tunstall, 1922) to be parasitic chiefly on plants weakened by some unfavourable condition of the environment. The changes brought about in the soil environment by rising moisture content are many and various, however, and it is impossible to generalize (Russell, 1937). In addition, high soil moisture content may be correlated with changes in the external environment favouring disease, in the occurrence, for instance, of conditions favouring aerial spore dispersal. Again, it has been suggested by Wardlaw (1935) that the

Table II. *Diseases favoured by low soil moisture content*

Fungus	Disease	Authorities
<i>Actinomyces poolensis</i>	Pox of sweet potatoes	Poole (1925a)
<i>A. scabies</i>	Potato scab	Millard (1923), Sanford (1923)
<i>Sorosporium reilianum</i>	Head smut of sorghum	Christensen (1926)
<i>Sphacelotheca sorghi</i>	Covered smut of sorghum	Reed & Faris (1924a)
<i>S. cruenta</i>	Loose smut of sorghum	Reed & Faris (1924a)
<i>Tilletia tritici</i>	Bunt of wheat	Gibs (1924), Rabien (1927)
<i>T. levis</i>		
<i>Urocystis tritici</i>	Flag smut of wheat	Faris (1933)
<i>Ustilago avenae</i>	Loose smut of oats	Reed & Faris (1924b), Johnston (1927)
<i>U. levis</i>	Covered smut of oats	Reed & Faris (1924b), Johnston (1927)
<i>U. hordei</i>	Covered smut of barley	Rump (1926)
<i>Fusarium hyperoxysporum</i>	Stem rot of sweet potatoes	Poole (1924)
<i>F. batatis</i>		
<i>Gibberella saubinetii</i>	Seedling blight of wheat and corn	Dickson <i>et al.</i> (1923)

Panama disease of bananas may be especially prevalent in moist, low-lying situations largely because such areas are subject to periodical flooding, and hence to dissemination of the causal organism by water.

Certain diseases, on the other hand, are favoured by low soil moisture content (Table II). Both the *Actinomyces* spp. and the smut fungi, of which latter nine are given above, are known to be strongly aerobic, and a good supply of oxygen is essential for spore germination and infection of the host, as has been shown by Sanford (1926) for *A. scabies* and by Rabien (1927) and others for the smut fungi. Low soil moisture content is thus considered to favour these diseases, in part at least, through the promotion of better aeration conditions favourable to the parasite. At the same time, low soil moisture content may also favour smut infection by retarding the germination and early growth of the cereal seedlings, whilst Dickson *et al.* (1923) have clearly shown that the *Gibberella saubinetii* seedling blight of wheat and corn is more serious in the drier soils owing to retarded development of the natural host resistance.

(2) *Soil texture*

Heavy soils appear to be favourable to few diseases (Table III). In the case of *Cercospora herpotrichoides*, heavy soils, and especially those rich in nitrogen, appear to induce a soft, susceptible type of growth in the wheat plant (Oort, 1936).

Table III. *Diseases favoured by heavy soils*

Fungus	Disease	Authorities
<i>Cercospora herpotrichoides</i> <i>Calonectria graminicola</i> <i>Sclerotinia graminearum</i> <i>Sphaerostilbe repens</i>	Eye-spot disease of cereals Snow mould of cereals Snow mould of cereals Violet root rot of tea	Oort (1936) Korff (1924), Laube (1926) Khokhryakoff (1935) Tunstall (1922), Pinching (1925)

This may also be so with *Calonectria graminicola* and *Sclerotinia graminearum*; these diseases are also favoured by a high soil moisture content, however, and on heavy soils the water from the melting snow would be retained for a longer period than on the lighter soils (Eleneff, 1926). Invasion of the host roots by *Sphaerostilbe repens* appears to be especially favoured by poor aeration, such as might be associated with excessive moisture and with heavy soils, in lowering host resistance (Tunstall, 1922).

Diseases reported to be worst on soils of light texture are given in Table IV. It appears that the majority of soil-borne fungus diseases are favoured by soils of light texture. In every disease investigated, light soils appear to have favoured

Table IV. *Diseases favoured by light soils*

Fungus	Disease	Authorities
<i>Actinomyces scabies</i> <i>Botryodiplodia theobromae</i> <i>Fomes annosus</i> <i>F. lucidus</i> <i>F. noxius</i> <i>Fusarium cubense</i> <i>F. lini</i> <i>F. hyperoxysporum</i> } <i>F. batatis</i> } <i>F. orthoceras</i> var. <i>pisi</i> <i>F. vasinfectum</i> <i>Fusarium</i> sp. <i>Ophiobolus graminis</i> <i>Sclerotinia sclerotiorum</i> <i>S. trifoliorum</i> <i>Spongospora subterranea</i> <i>Tilletia tritici</i> } <i>T. levis</i> }	Potato scab Internal root rot of tea Red rot of pines Root rot of coconut palms Brown root rot of tea and rubber Panama disease of bananas Flax wilt Stem rot of sweet potatoes Pea wilt Cotton wilt Collar rot of peas Take-all of wheat Collar rot of lettuce Clover rot Powdery scab of potatoes Bunt of wheat	Millard (1923) Tunstall (1922) Anderson (1921), Falck (1930) Bryce (1924) Tunstall (1930), Peelen (1930) Reinking (1935) Bolley & Manns (1932) Poole (1924) Walker & Snyder (1934) Young (1928), Zaprometoff (1929) Moore (1923) Garrett (1936) Soursac (1922) Pape (1931) Wild (1929) Gassner (1925)

infection chiefly because they seemed to provide an environment especially suitable for the activity of the pathogenic fungus. Thus *Actinomyces scabies* has been shown by Sanford (1926) to be very susceptible to the aeration conditions of the medium, and hence to be favoured by low soil moisture content and soils of light texture. Gassner (1925) and Rabien (1927) demonstrated that spore germination in *Tilletia*

tritici and *T. levis* closely paralleled infection inasmuch as both were favoured by the good aeration conditions obtaining in soils of low moisture content, and in soils of light texture. In pure clay, no infection occurred. The heavy incidence of flax wilt on light soils was shown by Bolley & Manns (1932) to be correlated with exceptionally high numbers of the causal organism in such soils. The correlation between soil type, numbers of the causal organism in the soil, and severity of the disease has been well brought out by the studies of Reinking (1935) on the Panama disease of bananas. Counts of *Fusarium cubense* in soil adjacent to diseased plants gave good correlation both with severity of the disease and with the soil type, numbers increasing in a regular manner with increasing percentage of sand and decreasing percentage of clay in the soil. Reinking concluded that the severity of the disease was chiefly determined by the numbers of the causal organism in the soil, and that the effect of soil conditions upon host resistance was not of much importance. In the take-all disease of wheat, good correlation was found by Garrett (1936) between those soil conditions favouring the most rapid growth of *Ophiobolus graminis* along the roots, and those most favourable to the occurrence of the disease in the field.

(3) Soil organic matter

In a number of diseases, the addition of organic manures to the soil has been found to exercise a controlling effect (Table V). The control of fungus root diseases by the application of organic matter to the soil has been attributed to the antagonistic action of other soil organisms developing on the added organic material.

Table V. *Diseases controlled by application of organic matter*

Fungus	Disease	Authorities
<i>Actinomyces scabies</i>	Potato scab	Sanford (1926), Millard & Taylor (1927)
<i>Fusarium lini</i>	Flax wilt	Bolley & Manns (1932)
<i>F. vasinfectum</i>	Pigeon-pea wilt	McRae & Shaw (1933)
<i>Ophiobolus graminis</i>	Take-all of wheat	Fellows (1929)
<i>Phymatotrichum omnivorum</i>	Cotton root rot	King <i>et al.</i> (1934a)

The control of potato scab by green manuring was shown by Millard & Taylor (1927) to be due to the antagonistic action of saprophytic species of *Actinomyces* and other soil micro-organisms, which were encouraged by the addition of the organic matter. The very striking control of cotton root rot obtained in field trials over a period of 13 years by the use of organic manures was attributed by King, Hope & Eaton (1934a) to the operation of this factor. These authors supported their hypothesis by an extensive study of the soil microflora in the two series of plots by the Cholodny slide technique (Eaton & King, 1934). By this method, they found that whereas the development of saprophytic organisms was most profuse on the slides buried in the manured plots, the mycelium of *Phymatotrichum omnivorum* was most abundant on the slides buried in the unmanured plots. Although the number of soil-borne diseases thus far found to be controllable by

application of organic matter is very limited, it appears likely that further investigation will greatly extend this list. For if it be true that organic matter gives control chiefly by promoting biological antagonism to the parasite, then this principle should be one of wide application.

Certain diseases, on the other hand, are undoubtedly favoured by the application of organic matter to the soil (Table VI). At one time, it was widely considered that organic matter might increase the incidence of a soil-borne fungus disease by promoting the multiplication of the pathogen on the added organic material (Bewley, 1923). This is probably true only of a very limited number of fungi, e.g. species of *Rosellinia*.

Even though not actually permitting multiplication of a parasite, however, organic matter may yet exercise a stimulating effect on its activity. This appears to be so with some, at least, of the smut fungi. Thus infection by *Tilletia tritici* and *T. levis*, *Urocystis tritici* and *U. zeae* is favoured by the presence of organic matter in the soil. Spore germination in *Tilletia tritici* and *T. levis* was stimulated by the presence of humus material (Rabien, 1927), and in *Urocystis tritici* by various

Table VI. *Diseases favoured by application of organic matter*

Fungus	Disease	Authorities
<i>Tilletia tritici</i> }	Bunt of wheat	Rabien (1927)
<i>T. levis</i> }		
<i>Urocystis tritici</i>	Flag smut of wheat	Forster & Vasey (1929)
<i>Ustilago zeae</i>	Corn smut	Borzini (1935)
<i>Verticillium albo-atrum</i>	Tomato wilt	Bewley (1922), Brittlebank (1924)
<i>Rosellinia arcuata</i>	Root rot of tea	Tunstall (1922), Petch (1923)
<i>R. bunodes</i>	Root rot of tea	Tunstall (1922), Petch (1923)

organic substances in low concentration (Noble, 1924). Platz *et al.* (1927) found that crushed plant tissue encouraged the germination of spores of *Ustilago zeae* in closed chambers, and that this stimulating effect was due to the carbon dioxide given off; an atmospheric concentration of 15 per cent carbon dioxide was found optimum for spore germination. Lundegårdh (1923) observed that a concentration of carbon dioxide not exceeding 7 per cent in the atmosphere increased both the growth of *Fusarium culmorum* and *Gibberella saubinetii* in culture, and the production of seedling blight in wheat by these organisms. The latter effect was attributed in part, however, to the deleterious effect of carbon dioxide on the germination and early growth of the wheat seedlings. Lundegårdh considered that this effect of carbon dioxide in promoting infection largely explained the stimulating effect of fresh organic manure on the *Fusarium* foot rots of cereals, recently commented upon by Schmidt & Feistritz (1933).

The application of organic matter, however, may profoundly modify the soil environment in a variety of ways, for a discussion of which reference may be made to Russell (1937). Thus whereas the application of well rotted organic matter to the soil was found by Fellows (1929) to give good control of the take-all disease of wheat, Åkerman *et al.* (1935) have reported that the application of fresh, insufficiently rotted stable manure encouraged the disease. Fresh stable manure

opens up the soil, however, on account of the undecomposed straw residues, and the improvement in soil aeration thus effected would certainly encourage the activity of the take-all fungus. Again, in other cases, the use of organic material containing infected plant residues may serve to introduce or to augment infection. The use of infected "dessa" manure is stated by Thung (1932) to constitute a serious source of black shank infection on the tobacco fields of Java.

(4) Soil reaction

Certain diseases are reported to be favoured by acid soils (Table VII); others, again, are more serious in alkaline soils (Table VIII). The effects of soil reaction, both upon the activity of the causal fungus and upon the resistance of the host plant are probably complex, and our knowledge of the physiology of parasitism in

Table VII. *Diseases favoured by acid soils*

Fungus	Disease	Authorities
<i>Armillaria mellea</i>	Mushroom root rot	Gard (1928), Reitsma (1932)
<i>Botryodiplodia theobromae</i>	Internal root rot of tea	Tunstall (1929)
<i>Fomes annosus</i>	Red rot of pines	Anderson (1921), Falck (1930)
<i>Fusarium congenitans</i> var. <i>callistephi</i> and var. <i>majus</i>	Wilt of china asters	Wager (1932), Voglino (1932)
<i>Fusarium oxysporum</i> var. <i>nicotianae</i>	Tobacco wilt	Johnson (1921)
<i>F. lycopersici</i>	Tomato wilt	Sherwood (1923), Scott (1926)
<i>F. vasinfectum</i>	Cotton wilt	Taubenhaus <i>et al.</i> (1928)
<i>Plasmodiophora brassicae</i>	Club root of crucifers	Wellman (1930)
<i>Spongospora subterranea</i>	Powdery scab of potatoes	Janchen (1921), Blatný (1935)
<i>Synchytrium endobioticum</i>	Potato wart	Némec (1935)
<i>Ustilago hordei</i>	Covered smut of barley	Faris (1924)
<i>Ustilina zonata</i>	Charcoal root rot	Tunstall (1922)
<i>Aphanomyces levii</i>	Root rot of sugar-beet	Arrhenius (1924)
<i>Phoma betae</i>		
<i>Pythium de Baryanum</i>		

Table VIII. *Diseases favoured by alkaline soils*

Fungus	Disease	Authorities
<i>Actinomyces poolensis</i>	Pox of sweet potatoes	Poole (1925b)
<i>A. scabies</i>	Potato scab	Millard (1923)
<i>Calonectria graminicola</i>	Snow mould of cereals	Schaffnit & Meyer-Hermann (1930)
<i>Fusarium orthoceras</i> var. <i>pisi</i>	Pea wilt	Starr (1932)
<i>Monilochaetes infusans</i>	Scurf of sweet potatoes	Poole (1925b)
<i>Ophiobolus graminis</i>	Take-all of wheat	Garrett (1936)
<i>Phymatotrichum omnivorum</i>	Cotton root rot	Taubenhaus <i>et al.</i> (1928), Ezekiel <i>et al.</i> (1930)
<i>Thielavia basicola</i>	Black root rot of tobacco	Morgan & Anderson (1927), Doran (1931)
<i>Urocystis tritici</i>	Flag smut of wheat	Forster & Vasey (1929)
<i>Verticillium albo-atrum</i>	Tomato, etc., wilt	Haenseler (1928), Martin (1931)

these soil-borne diseases is not sufficiently advanced for generalization, or even detailed comment. Thus Garrett (1936) has shown that whereas the growth of *Ophiobolus graminis* along the host root is accelerated by an alkaline reaction of the soil, growth of the fungus on the surface of sterilized soil shows little relation to soil

reaction. The need for further study of the mechanism of fungal growth and parasitism in the actual soil environment is thus emphasized. In view of work on the physiology of parasitism by Brown and his collaborators, recently summarized (Brown, 1936), it seems possible that in some soil-borne diseases soil reaction may, indirectly perhaps, affect the enzymic mechanism of infection by the fungus. Thus Weindling (1934*a*) has shown that the "lethal principle" excreted by *Trichoderma lignorum*, by which it is enabled to parasitize other soil fungi, is comparatively stable only under acid conditions, being rapidly decomposed in alkaline media. Finally, in view of the very general observation (Waksman, 1931) that the saprophytic soil fungi attain their greatest development in acid soils, it is certainly interesting to note that so many important soil-borne diseases of different types are favoured by alkaline soils.

(5) *Chemical composition of the soil*

Recent studies by Vanterpool (1935) on browning root rot of cereals due to *Pythium arrhenomanes* in Canada, and by Carpenter (1934) on the root rot of sugar-cane caused by the same species (Drechsler, 1936) in Hawaii, have revealed an interesting correlation between the nitrogen/phosphorus ratio of the soil and the incidence of disease. A high nitrogen/phosphorus ratio is considered to modify host resistance in such a way as to favour infection by these organisms, which cause much less apparent injury to the host, though yet abundantly present, in soils of lower nitrogen/phosphorus ratio. The term host resistance is here used in its widest sense, since it appears from the work of Vanterpool (1935) that the beneficial effect of superphosphate in controlling browning root rot of cereals is to be largely assigned to the increase in rooting capacity which it confers on the cereal plant, and that the influence of additional phosphate on the resistance of individual roots is not very apparent. This disease is of particular interest in that it is much more serious in crops sown after a fallow than in those sown on stubble land, contrary to experience with other cereal root rots, and, indeed, with soil-borne diseases in general. This has been attributed by Vanterpool to the increase in the available nitrogen/phosphorus ratio of the soil as a result of accumulation of nitrate during the fallow. Similarly, certain other diseases, such as take-all of wheat, may be controlled to some extent by the application of superphosphate. On the other hand, McRae & Shaw (1933) found that superphosphate markedly increased the incidence of pigeon-pea wilt due to *Fusarium vasinfectum* in field trials over a period of years in India. Again, whereas the *Pythium* root rots of sugar-cane and cereals are aggravated by applications of nitrogen, root rot of peas due to *Aphanomyces euteiches* has been reported by Martin (1934) and by Geach (1936) to be controlled by nitrogenous manures, which control Geach, on the basis of laboratory experiments, has attributed to a directly inhibiting effect of excess nitrogen on the reproduction and survival of the causal fungus, *A. euteiches*.

IV. SUMMARY

An examination has been made of papers published during the last 15 years on soil-borne fungus diseases of plants, with especial reference to the influence of soil conditions on infection.

In reviewing the ecology of the root-infecting fungi, a distinction has been made, following Reinking, between *soil inhabitants* and *soil invaders*.

The *soil inhabitants* are considered to be primitive or unspecialized parasites with a wide host range; these fungi are distributed throughout the soil, and their parasitism appears to be incidental to their saprophytic existence as members of the general soil microflora.

The *soil invaders*, to which class the majority of the root-infecting fungi seem to belong, are more highly specialized parasites; the presence of such fungi in the soil is generally closely associated with that of their host plants. In the continued absence of a host plant, such fungi die out in the soil, owing to their inability to compete with the soil saprophytes for an existence on non-living organic matter. This close association between the *soil invaders* and their host plants thus seems to be enforced by the competition of the general soil microflora.

The influence of soil conditions upon a number of soil-borne fungus diseases has been tabulated and discussed under the headings of soil moisture content, texture, organic matter, reaction and chemical composition. The relation of soil temperature to soil-borne diseases has already been reviewed at length by L. R. Jones *et al.* (1926) and has therefore not been further considered here.

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ADDENDUM

Since this article went to press, an important contribution to the subject has been published by R. Leach (1937) (*Proc. roy. Soc. B*, **121**, 561) concerning *Armillaria mellea* in Nyasaland. This fungus normally occurs to a moderate extent as a parasite on the jungle roots, but after the trees have been felled, the activity of *A. mellea* greatly increases, and the roots of almost every stump of certain species of tree may become infected by the fungus. A similar observation has been made by G. Wallace (1935) (*Rep. Dep. Agric. Tanganyika*, 1934, p. 90) in Tanganyika; the increase in development of *A. mellea* can probably be attributed to the lowering of root resistance after felling. Whilst the fungus thus becomes widespread upon the roots of mature jungle trees only after felling, it behaves, on the other hand, as a serious parasite of the young tea bushes planted upon the cleared land.

Leach has made the significant observation that when the jungle trees are ring-barked some time before felling, the roots are generally invaded not by *A. mellea*, but by other fungi, such as *Rhizoctonia bataticola* and *Botryodiplodia theobromae*, which appear to be harmless in tea plantations. On the basis of field observations and microscopical studies, it is concluded that *A. mellea* can only invade and parasitize roots with a normally high carbohydrate content; if this is seriously depleted by the act of ring-barking the trees some time before felling, the roots are invaded by other fungi instead. The relation of these observations to the control of *A. mellea* in newly established tea plantations is discussed.

Leach's findings may be considered in logical sequence to the earlier work of Gadd (1928-29) (*Tea Quart.* **1**, 89, and **2**, 54) on the "disease" formerly known as *Botryodiplodia theobromae* root rot of tea in Ceylon. This "disease" occurs in Ceylon chiefly at mid- and low elevations, where the plucking rounds are most frequent, and the pruning-cycle the shortest; it is manifested as a failure to make fresh growth after pruning, and by subsequent death of the bush. Gadd showed that failure to recover after pruning appeared to be invariably associated with absence of starch reserves in the roots, and that this condition preceded infection by *Botryodiplodia*. Lack of starch reserves thus appeared to be the cause, and the general subsequent infection of the weakened roots by *Botryodiplodia* merely an effect of the disease. In tea grown at the higher elevations, where assimilation could better keep pace with growth, the "disease" was almost unknown. Gadd also observed that whereas roots infected by *Botryodiplodia* were completely deficient of reserve starch, those infected by *Poria*, *Rosellinia* and *Ustilina* showed a normal starch content. The apparently contrary observation of Tunstall (1929) (*Quart. J. Indian Tea Ass.* for 1929, 68) in North East India of invasion by *Botryodiplodia* of tissues with a normal starch content appeared to apply to this ubiquitous fungus acting in another role, that of a wound-invader via stem wounds.

The practical implications of this and other recent work on the root-infecting fungi have been discussed elsewhere by Garrett (1937) (*Emp. J. exp. Agric.* **5**, 189) in a review of control methods.

FACTORS INVOLVED IN THE PROCESS OF ORIENTATION OF LOWER ORGANISMS IN LIGHT

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I. INTRODUCTION

THE fundamental aim of biology is to understand the processes that occur in living organisms so as to be able to control their behaviour and their development. Orientation and movement in reference to definite regions in space are among the most important phenomena in the behaviour of all organisms, for they have to do with the control of environment no matter whether they consist of merely the turning of the leaf toward the light or the migration of a bird or the gathering of men at an epoch-making convention. The processes involved in these responses are consequently of fundamental importance and they have been seriously investigated for many years.

Early in the history of the subject it was generally held that orientation in all organisms depends upon the action of vital forces, psychic phenomena and the like. This attitude continued till well beyond the middle of the nineteenth century in reference to animals, but it was abandoned much earlier in reference to plants.

Ray (1693) was probably the first to offer a mechanical explanation of orientation in any organism. He maintained that plants in a window turn toward the light be-

cause the side toward the window is colder than the opposite side and consequently does not grow so rapidly, and that the difference in the rate of growth on opposite sides causes bending toward the window. De Candolle (1832) gave precisely the same explanation as Ray except that he held that the difference in rate of growth is due to difference in illumination of opposite sides in place of difference in temperature. According to this explanation, the motor and the receptor tissues are not differentiated, the motor tissues are stimulated directly and the stimulating agent acts continuously; and orientation is dependent upon the relative activity of the tissues on opposite sides of the reacting organ and this is specifically related to the relative amount of stimulating energy received by these tissues.

Sachs (1882, p. 851) rejected this explanation. He maintained that orientation in plants is in some unknown way dependent upon the *direction in which the stimulating energy passes through the tissues* ("die Pflanzensubstanz durchsetzt"), not upon the relative amount received on opposite sides of the reacting organ.¹

Darwin (1880) proved that the receptor tissues in plants are separated from the motor tissues, and that organs of plants move continuously; and he concluded that orientation is due to modification of this movement, owing to impulses which pass from the receptor to the motor tissues when the organs are laterally acted upon by stimulating agents, and that orienting stimulation is primarily dependent upon rate of change of intensity of these agents.

Up to some ten years after the publication of the *Origin of Species* by Darwin (1859) the main object of those interested in the behaviour of animals was to inculcate humane treatment of animals. This resulted in the publication of numerous books consisting primarily of collections of hearsay accounts of episodes concerning animals, e.g. Menault's *Wonders of Animal Instinct*, five editions; Jesse's *Anecdotes of Dogs*; Swainson's *Habits and Instincts*; Cough's *Instincts*, etc. The publication of the *Origin of Species* and the comparative embryological, anatomical and palaeontological investigations which followed, clearly demonstrated that plants and animals, including man, are structurally interrelated. This convinced a considerable number of investigators that psychic processes in man had their origin in the lower organisms and led them to investigate the behaviour of these organisms with the purpose of obtaining evidence in support of this conviction. With this in view Bert (1869) investigated "La question de savoir si tous les animaux voient les mêmes rayons que nous"; Darwin (1872), *Expression of the Emotions*; Lubbock (1881), "The sense of colour among some of the lower animals"; Graber (1883), "Die Helligkeits- und Farbenempfindlichkeit augenloser und geblendeter Thiere"; Romanes (1883), *Animal Intelligence*, and Binet (1889), *The Psychic Life of Micro-organisms*. These men were obviously interested in the question as to whether or not there are psychic processes in the lower animals, not in the questions concerning the mechanics of responses in them. There were, however, some investigators who in this period were interested in both, notably Engelmann (1879) and Verworn (1889); and at least one, Jacques Loeb (1890), who was interested only in the latter.

Engelmann made very precise observations on various types of responses in the

¹ Sachs first expressed these views in the preface of a paper by H. Müller published in 1876.

lower animals, but he did not express any views concerning the process of orientation. Verworn (1895) did however, and he accepted, in principle, the Ray-Candolle theory of orientation and applied it to the lower animals. Loeb (1888), on the other hand, rejected this theory and accepted the Sachs ray-direction theory. He says: "Die Orientierung der Thiere gegen eine Lichtquelle wird wie bei den Pflanzen (J. v. Sachs) bedingt durch die Richtung in welcher die Lichtstrahlen die thierischen Gewebe durchsetzen, und *nicht durch die Unterschiede in der Lichtintensität auf den verschiedenen Seiten des Thieres.*" (Italics mine.) Loeb continued to support the Sachs theory in various modified forms until about 1906, when he appears definitely to have abandoned it in favour of the Ray-Candolle theory as applied to animals by Verworn. He says (1906, p. 130) orientation is determined by the relation of the intensity of light on the photosensitive elements on opposite sides (1913, p. 463): "Wenn nun ein Tier seitlich vom Licht getroffen wird, so wird eine Hälfte des Nervensystems in stärkeren 'Phototonus' geraten als die andere. Wenn bei einem solchen Tiere Impulse zu einer Lokomotion stattfinden, so wirken die Impulse nicht wie gewöhnlich auf beide Seiten des Tieres in gleicher Weise, sondern die mit beiden Hirnhälften verbundenen Muskeln werden verschieden stark arbeiten" and (1918, p. 83): "Motile plant organisms like *Volvox*, are driven to the source of light, owing to differences in the tension of the contractile organs on the shaded and illuminated side, and the same is true for animals like insects." Loeb held that orientation is fundamentally non-adaptive and that it originated by chance occurrence of bilateral symmetry and photosensitive substance in the same organism.

The fundamental principles of the Ray-Candolle theory have since been accepted by many. Among these Bohn and Patten made the most specific declarations. Bohn says (1909, p. 9): "The side which is more strongly stimulated by the light moves more rapidly than the other (*marchera plus vite que l'autre*), and this results in a forced turning movement"; (1909 *a*, p. 5): "Orientation (*tropisme*) presents itself to us as the forced result of the inequality of the same activities in the right and the left half of the body, an inequality which is purely quantitative, not qualitative. In other words, orientation (*tropisme*) is not an activity (*une activité*), it is the result of an inequality between certain activities, simple or complex, which occur on one side of the body and the same activities which occur on the opposite side." Patten says (1919, p. 457): "Orientation is attained and maintained by a transmission of impulses to the muscles of locomotion which is proportional bilaterally to the excitation of the symmetrically located photo-receptors." It "depends upon bringing the excitation of the receptive mechanism as a whole into bilateral equilibrium".

According to this theory then orientation in animals is the result of a balanced action of the locomotor appendages on opposite sides just as in a row boat which has no rudder and is propelled and guided with oars, the rate of movement in the appendages is directly proportional to the amount of light received by the receptors connected with them; the processes involved are not dependent upon the location of the stimulus in the receptors, the stimulating agent acts continuously, not intermittently, and the orienting stimulus continues after the organism is oriented as well as during the process of orientation. That is, the essential factors involved are (1)

bilateral symmetry of the organism; (2) continuous action of the stimulating agent in direct proportion to the amount of energy received by the receptors on opposite sides; (3) quantitative (not qualitative) difference in the action of the locomotor mechanism (appendages or tissues) on opposite sides directly proportional to the difference in the amount of energy received by the receptors on opposite sides during the process of orientation; (4) continuous action of the stimulating agent after orientation, resulting in equal action of the locomotor mechanism on opposite sides and consequently in direct movement.

Views concerning orientation which are in all essentials in accord with these characteristics have been designated "the difference of intensity theory", "the continuous-action theory", "the tropism theory", "Loeb's tropism theory", "the Verworn theory", "the muscle-tonus theory", and "Loeb's muscle-tension theory". I shall refer to them as the Ray-Verworn theory.

Jennings (1904, 1906) observed numerous phenomena associated with the process of orientation in animals which are not in accord with the Ray-Verworn theory. He concluded that, while orientation in some animals is doubtless direct, i.e. essentially in accord with the Ray-Verworn theory, random movement and retention of favourable axial positions (trial and error) play a predominant role in others. He holds that the random method is more primitive than the direct method and that the latter developed from the former by natural selection, i.e. he holds, in opposition to Loeb, that orientation is fundamentally adaptive.

Holmes (1905), Mast (1911), Buddenbrock (1915), Kühn (1919) and others agree with Jennings in the contention that the fundamental factors involved in the process of orientation are not the same in all animals. Kühn (1919, 1929) maintains that there are four fundamentally different methods of orientation and he proposed a name for each (see Fraenkel,¹ 1935). This classification has been accepted, at least in part, by Koehler (1931) and others in Germany but by no one elsewhere, as far as I know.

In a comprehensive study which extended over more than twenty years, I have attempted to ascertain precisely what factors are involved in the process of photic orientation in representative species of the different groups of animals from *Amoeba* to insects inclusive. I shall in the following pages devote myself largely to the presentation of the more important of the results obtained in this study.

II. RHIZOPODS

Many of the rhizopods respond to light and a considerable number orient. All are photonegative except perhaps in very low illumination. The process of orientation has been thoroughly studied in only one species, *Amoeba proteus*.

Davenport (1897, p. 186) exposed specimens of *Amoeba proteus* in a beam of direct sunlight and found that they orient fairly precisely and that if the direction of the rays is changed they turn directly from the light, but he did not ascertain the process involved.

¹ *Biological Reviews*.

Mast (1910) made detailed observations on the pseudopods during the process of orientation in a horizontal beam of direct sunlight. He did not find any indication of bending from the light in any of the pseudopods but he found that no new pseudopods were formed on the more highly illuminated side (Fig. 1). He concluded that turning is due to inhibition in the formation of pseudopods on the more highly illuminated side and he accounts for this as follows:

Amoeba proteus consists of a thin elastic outer membrane, the plasmalemma, a central relatively fluid granular mass, the plasmasol, and a thin fluid hyaline layer between the plasmagel and the plasmalemma. During locomotion the plasmalemma is attached to the substratum and to the adjoining plasmagel, the plasmagel at the

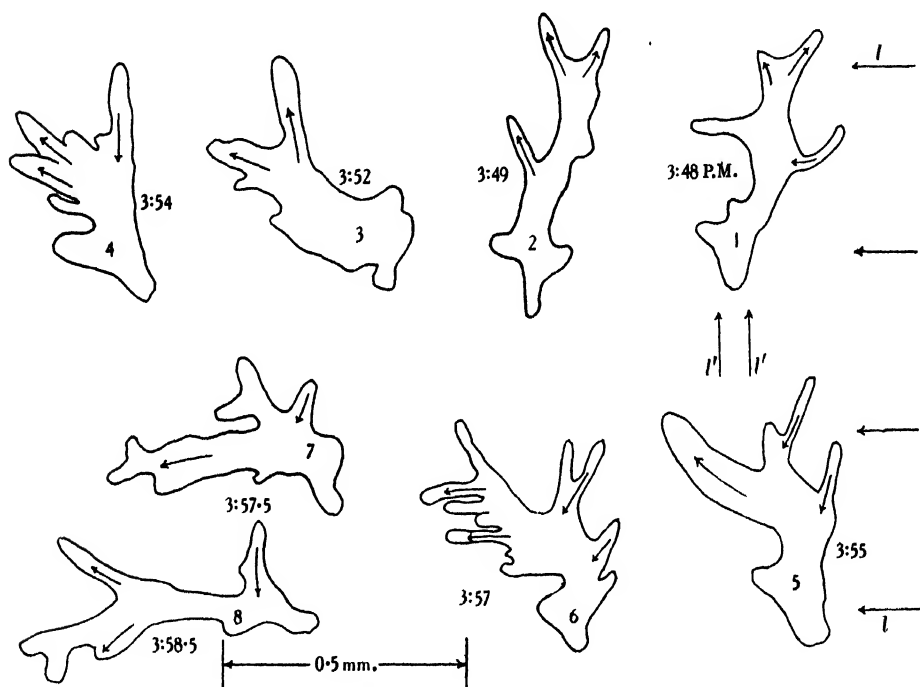


Fig. 1. Camera outlines representing different stages in the process of orientation in *Amoeba proteus*. 1, *Amoeba* oriented in light l' ; 2-8, successive positions after exposure to light l , time indicated in each. Arrows represent the direction of streaming of protoplasm in pseudopods. In those which do not contain arrows there was no noticeable streaming at the time the sketch was made. l and l' , direction of light. Projected scale in mm. (After Mast, 1910.)

posterior end is transformed into plasmasol which flows forward to the anterior end and is there transformed into plasmagel. The forward flow of the plasmasol surrounded by a relatively solid granular layer, the plasmagel is due to contraction of the plasmagel at the posterior end and expansion at the anterior end, owing to difference in its elastic strength in these two regions (Mast, 1926). Response in this form is due to changes in the elastic strength of localized regions in the plasmagel, or in the rate of transformation of plasmasol into plasmagel and *vice versa*, or in the attachment to the substratum.

The inhibition of formation of pseudopods on the more highly illuminated side must therefore be due either to increase in the elastic strength of the plasmagel on that side or to decrease on the opposite side.

Engelmann long ago (1879) observed that if the intensity of light is rapidly and strongly increased amoeboid movement ceases. This response was quantitatively investigated by Folger (1925) and it has been observed by many others. Mast (1931) found that it varies greatly, depending upon various conditions, that under some conditions it consists of merely a slight momentary local decrease in the rate of streaming and under others of total cessation in streaming throughout the entire organism and under still others of various series of phenomena, but that under all

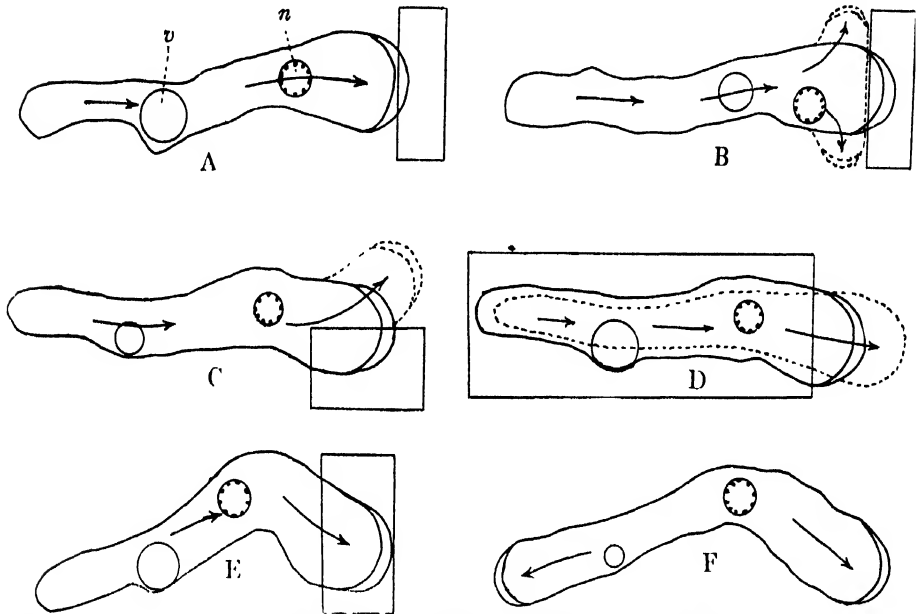


Fig. 2. Camera drawings of *Amoeba* (sp.), illustrating the response to localized illumination. Rectangular areas, regions of high illumination; arrows, direction of protoplasmic streaming; dotted lines in B, C and D, positions and forms shortly after the illumination of the parts indicated; n , nucleus; v , contractile vacuole. E and F, same specimen; F, form and direction of streaming assumed by E after the anterior end had been illuminated for a few minutes. (After Mast, 1932.)

conditions the response begins in decrease in the rate of flow at the tip immediately behind the hyaline cap. Adjoining the hyaline cap at the posterior surface there is during locomotion a very thin layer of plasmagel, the plasmagel sheet which continuously stretches as the plasmasol pushes against it in its forward flow. The fact that, in the response to increase in luminous intensity, retardation in flow always begins immediately behind the plasmagel sheet strongly indicates that the response is due to increase in the elastic strength of this sheet, that this is due to gelation of the plasmasol adjoining it, resulting in increase in thickness and that the gelation is produced by the action of the light (Fig. 2). The conclusion that increase in illumination produces gelation of the plasmasol adjoining the plasmagel sheet is strongly supported by the fact that if the increase in illumination is so great that the direction of

streaming after recovery is reversed, it can readily be seen that, immediately after the reversed flow begins, the plasmagel sheet is very much thicker than it was before the increase in illumination. This has been very clearly demonstrated by Luce (1926). Light not only causes increase in the thickness of the plasmagel at the tip of advancing pseudopods. This is demonstrated by the result of measurements which Mast (1932) made on the plasmagel in the central portions of a number of specimens before and after illumination. Retardation in the formation of pseudopods on the more highly illuminated side of amoebae resulting in orientation, is therefore in all probability due to increase in thickness and in the elastic strength of the plasmagel on this side. This would result in turning until both sides are equally illuminated, after which pseudopods would form equally readily on both sides and movement would continue directly from the source of light. If this is true the process of orientation in *Amoeba proteus* is obviously not in full accord with the Ray-Verworn theory. How light facilitates gelation of the plasmasol is not known. Folger (1926, 1927) maintains that mechanical stimulation causes cessation in movement in *Amoeba* which appears, point for point, to involve the same processes as cessation produced by increase in illumination, and that if mechanical stimulation too weak to produce a perceptible effect is followed by increase in illumination too slight to produce by itself a perceptual effect, or *vice versa*, there is cessation in movement. He concludes that light and mechanical agitation produce the same kind of changes, and that since the latter cannot produce photochemical changes, response to the former is not due to photochemical processes.

III. FLAGELLATES

Photic orientation is common in flagellates but the process has been thoroughly studied only in *Euglena*.

Nearly all species of *Euglena* respond to rapid changes in intensity of the light by abrupt changes in rate and in direction of movement, and if they are exposed in a beam of light nearly all orient and go either toward or from the light, i.e. they may be either photopositive or photonegative.

Verworn (1895) postulated that when *Euglena* is not oriented and opposite sides are unequally illuminated, the flagellum beats more effectively in one direction than in the other, that this results in turning until opposite sides are equally illuminated, and that the flagellum then beats equally in opposite directions, and the organism continues on a straight course.

Jennings (1904) contends that the process of orientation in *Euglena* consists essentially of random movements, resulting in the assumption of numerous axial positions, and retention of the most favourable of the axial positions assumed; and he holds that this obtains for photopositive specimens when the anterior end is directed toward the light and for photonegative specimens when it is directed from the light. Engelmann (1882) demonstrated that the photosensitive substance in *Euglena* is largely if not entirely confined to the anterior end. This substance is therefore most highly illuminated when *Euglena* faces the light, and consequently turning in any direction from this position results in decrease in the illumination of it. When

Euglena swims it rotates on the longitudinal axis and proceeds on a spiral course, and if the axis of this course is not parallel with the incident rays of light, the anterior end is, owing to the rotation and the spiral course, alternately more highly and less highly illuminated. Jennings maintains that this change in illumination stimulates the organism and causes it to turn until the axis of the spiral course is directed toward the light, when the change in illumination and stimulation cease.

Mast (1911) made a very intensive study of the process of orientation in a species of *Euglena* which crawls on the substratum. This *Euglena* orients very precisely in light, it has a well-developed eyespot and it moves so slowly that the different phases of its responses can readily be followed in detail. It is therefore very favourable for the study of the process of orientation.

If the intensity of the light is rapidly decreased in a beam in which specimens are proceeding toward the source of light they stop suddenly and bend in the middle toward the abeyespot surface until the two halves form nearly a right angle; then they begin to rotate on the longitudinal axis again and as they rotate they gradually straighten and start to crawl toward the light again. If the intensity of the light is increased or if it is slowly decreased there is no perceptual response. The cessation of movement and the bending is therefore dependent upon the rate of decrease in the intensity of the light in the field, i.e. it is a shock-reaction. The decrease in the intensity of light in the field necessarily results in decrease in intensity of light on all the substance in the field; it therefore must cause decrease in the illumination of the photosensitive substance. The response then is dependent upon the rate of decrease in the light on the photosensitive substance.

If, without any change in the intensity of the light in the beam, the direction of the rays is changed through 90° so as to illuminate laterally the specimens oriented in it, those in which the eyespot surface faces the light after the direction of the rays has been changed, stop at once, bend in the middle toward the abeyespot surface, then rotate and gradually straighten, and soon begin to crawl again. Those in which the eyespot surface does not face the light, after the direction of the rays has been changed, do not respond until in the process of rotation on the longitudinal axis this surface faces the light, then they also stop, bend, rotate, straighten and proceed. Thus they continue until in the process of rotation the eyespot surface again faces the light, then they respond again. The gradual straightening during the rotation results in greater deflexion of the anterior end toward the light than from it. This end consequently gradually becomes directed more and more nearly toward the sources of light until an axial position is reached in which changes in illumination of the eyespot surface, owing to rotation, disappear and the organism is oriented (Fig. 3). Since the response which is induced by change in the direction of the rays, or by rotation in lateral illumination without change in the intensity of the light in the field, is precisely the same as the response induced by decrease in the intensity of the light in the field without change in the surface illuminated, change from illumination of the anterior end or the abeyespot surface to illumination of the eyespot surface must in some way result in rapid decrease in the illumination of the photosensitive substance. How is this brought about?

Wager (1900) demonstrated that the eyespot in *Euglena* consists of a spoon-shaped portion containing red pigment and a small globular enlargement of one of the roots of the flagellum in the concavity of the pigmented portion (Fig. 3). The

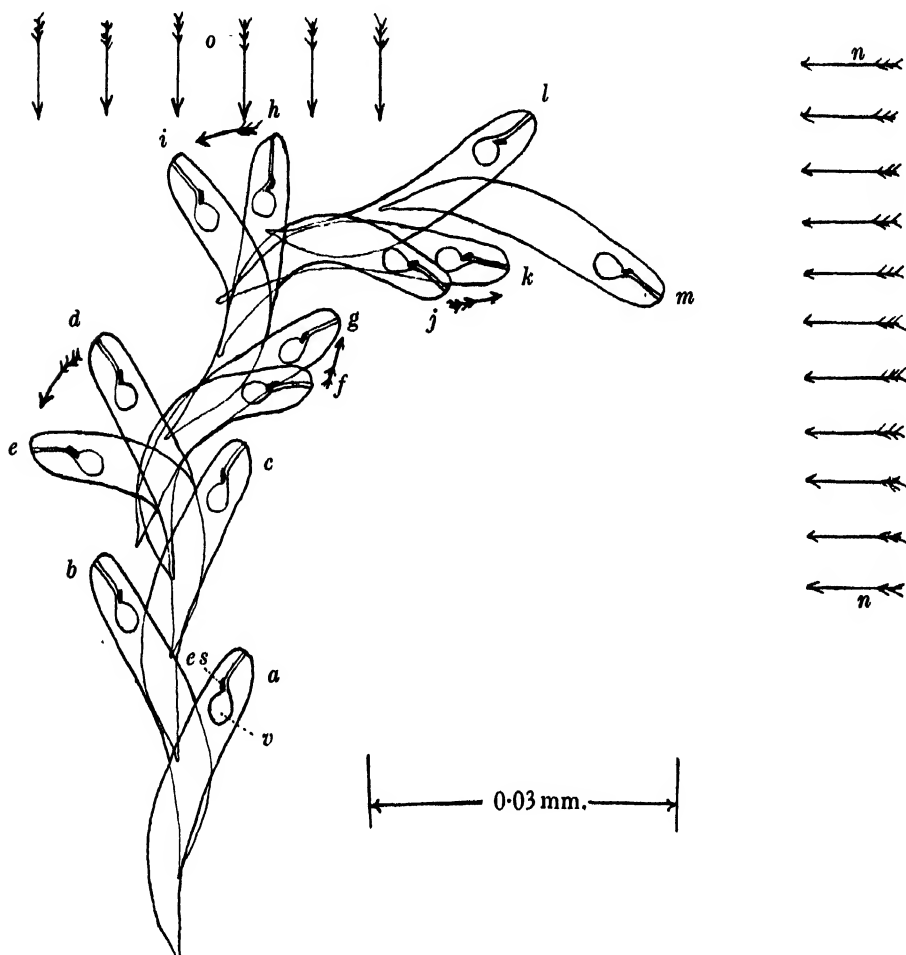


Fig. 3 a. *Euglena* (sp.) in crawling state, showing details in process of orientation; v, contractile vacuule; es, eyespot; n, o, direction of light; a-c, positions of *Euglena* with light from n intercepted; c-m, positions after light from n is turned on and that from o cut off so as to change the direction of the rays. If the ray direction is changed when the euglena is in position c there is no reaction until it reaches d. Then it suddenly reacts by bending away from the source of light to e, after which it continues to rotate and reaches position f, where it gradually straightens to g, and rotates to h, when the eyespot again faces the light and the organism is again stimulated and bends to i, from which it proceeds to j, etc. If the ray direction is changed when the euglena is at d, it responds at once and orients as described above. If the intensity from n is lower than that from o the organism may respond at once when the ray direction is changed no matter in which position it is. (After Mast, 1911.)

eyespot is situated near the eyespot surface a short distance from the anterior end with the convex surface directed outward and backward, so that when the anterior end or the abeyespot surface is directed toward the light the enlargement in the eyespot is fully exposed, and when the eyespot surface is directed toward the light the

enlargement is in the shadow cast by the pigmented portion. It is evident then that rapid change from illumination of the anterior end or the abeyespot surface to illumination of the eyespot surface causes rapid decrease in illumination of the enlargement in the eyespot, and that if the enlargement is photosensitive, change in the direction of the rays or rotation on the longitudinal axis has the same effect as decrease in the intensity of the light in the field. It is therefore highly probable that the enlargement in the eyespot is photosensitive and that the pigmented portion functions in producing changes in intensity of light on it, when the axial position of the organism changes and when it rotates on the longitudinal axis in lateral illumination. This contention is supported by the facts that the region of maximum

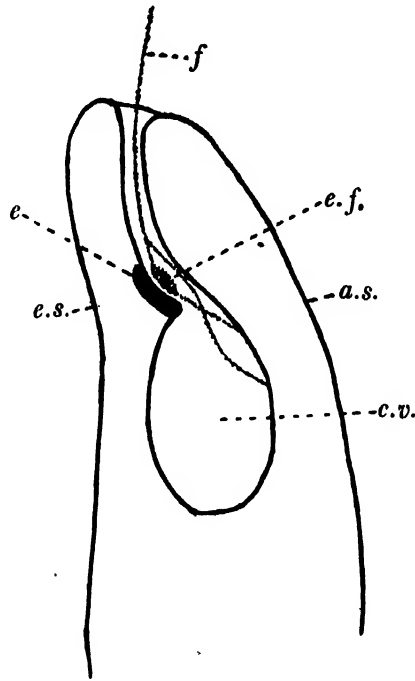


Fig. 3 b. Side view of anterior end of *Euglena viridis*. *e*, pigmented portion of eyespot; *f*, flagellum; *e.f.* enlargement in flagellum; *c.v.* contractile vacuole; *e.s.* eyespot surface of the organism; *a.s.* abeyespot surface of the organism. (After Wager, 1900.)

stimulating efficiency in the spectrum is in the blue for *Euglena* (Mast, 1917) and that this region is absorbed by the yellowish red pigmented portion of the eyespot.

In photonegative specimens the responses to changes in light intensity in the field and changes in the surfaces illuminated are precisely the same as they are in photopositive specimens except that the responses are induced by increase in place of decrease in intensity and by change from illumination of the eyespot surface to illumination of the abeyespot surface in place of the reverse.

Orientation in *Euglena* is then clearly due to a series of responses dependent upon the rate of change in the intensity of the light on the photosensitive substance, which is probably situated in the concave surface of the pigmented portion of the eyespot.

The light does not act continuously and there is no evidence whatever indicating anything in the nature of balanced or antagonistic action of locomotor appendages on opposite sides in accord with the Ray-Verworn theory.

The evidence in hand indicates, in short, that the photosensitive substance is confined to the concavity in the pigmented portion of the eyespot, that rotation on the longitudinal axis results in alternate shading and exposing of this substance, if the organisms are not directed toward or from the light, that this induces shock-reactions which result in orientation, and that the organisms remain oriented and proceed directly toward or from the light because, after they have attained either of these two axial positions, rotation no longer produces changes in the illumination of the photosensitive substance in the eyespot, and they therefore continue in the direction assumed, i.e. that the orienting stimulus ceases after the organism has become oriented and that it then continues directly toward or from the light because, owing to internal factors, it tends to take a straight course and because, if for any reason, it is turned from this course, the orienting stimulus immediately acts, and induces shock-reactions which bring it back on its course.

Bancroft (1913) presented evidence against the contention that photic orientation in *Euglena* is due to shock-reactions and concluded that it is due to tonus effects brought about by "the continuous action of the light" in accord with his conception of Loeb's tropism theory. Mast (1914) demonstrated, however, that if the evidence presented by Bancroft is valid, it proves that his explanation of orientation in *Euglena* is not correct. Moreover, the fact that after *Euglena* is oriented, the rate of locomotion is practically independent of the luminous intensity (Mast & Gover, 1922), also militates against his explanation.

If *Euglena* is subjected for long periods to low illumination or to darkness, it gradually becomes less active; and if the illumination is then increased it gradually becomes more active again. The rate of change in activity varies with the magnitude of the change in intensity, but this response is never so sudden and abrupt as the shock-reaction. There are therefore two types of responses to light in *Euglena*, one depending primarily upon the rate of change in luminous intensity, the other primarily upon change in the amount of light received. The one results in orientation and aggregation, the other in change in activity.

In a field of light consisting of two horizontal beams crossing at right angles, *Euglena* orients and goes toward or from a point between the two beams. The location of this point is related to the relative intensity of the two beams in such a way that the tangent of the angle between the direction of locomotion and the rays in the stronger beam is approximately equal to the intensity of the weaker divided by that of the stronger (Buder, 1917; Mast & Johnson, 1932). Buder judges this to show a quantitative proportionality between the stimulus and the response. Mast & Johnson conclude that "it has no bearing on the problem concerning the quantitative relation between stimulus and response", but that it can be explained on the assumptions that the eyespot is a photoreceptor and that the stimulating efficiency of light varies with the angle of incidence.

Euglena is in general photopositive in weak light and photonegative in strong

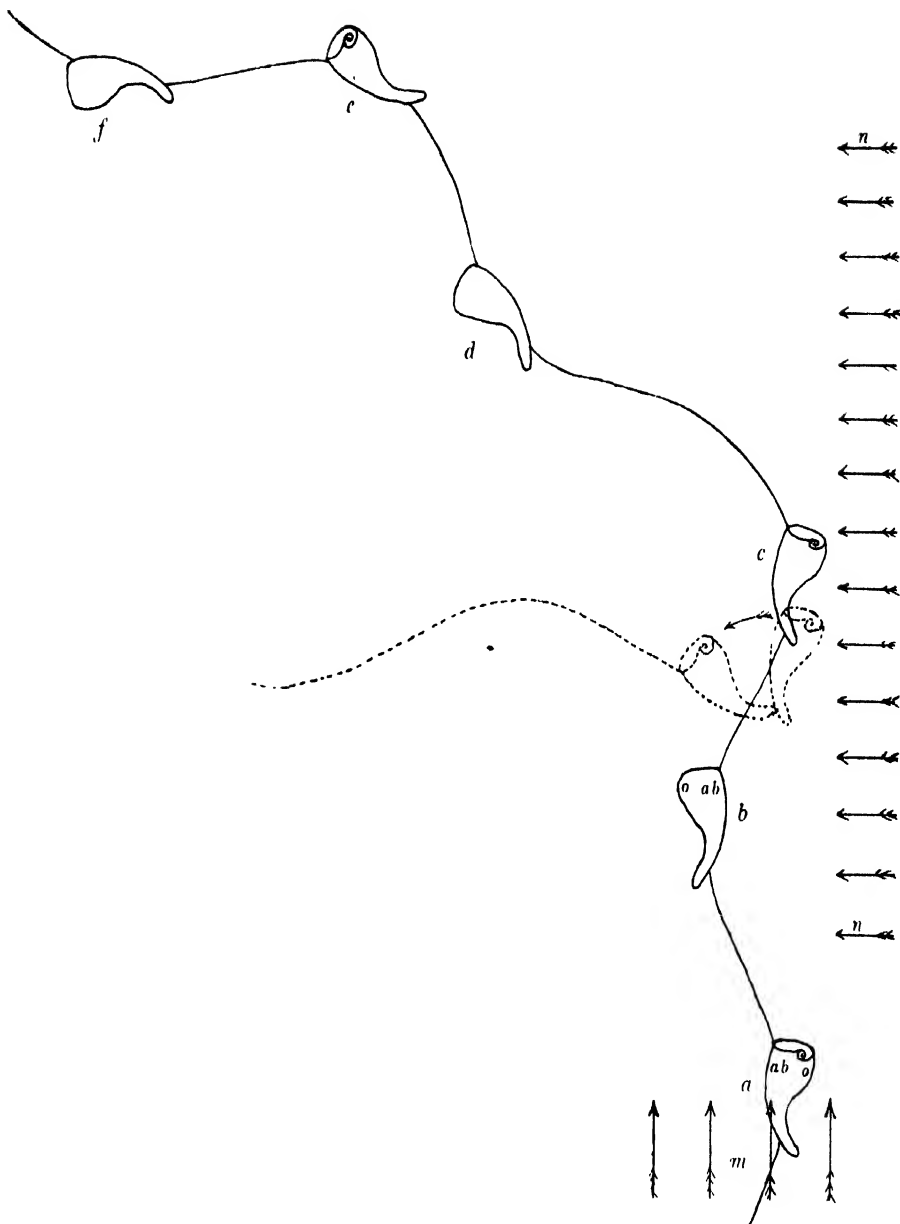


Fig. 4. *Stentor coeruleus* in the process of orientation. Curved line, spiral course; arrows *m* and *n*, direction of light from two sources; *a-f*, different positions of *Stentor* on its course; *o*, oral surface; *ab*, aboral surface. At *a* the *Stentor* is oriented in light from *m*, *n* being shaded. If *n* is exposed and *m* shaded simultaneously when the stentor is in position *b*, there is usually no reaction until it reaches *c* and the oral side faces the light; then the organism may respond by suddenly stopping, backing and turning sharply toward the aboral side (dotted outline), and become oriented at once; or it may merely swerve toward the aboral side without stopping. At *e* the oral side is again exposed and the organism is again stimulated and it again swerves from the source of light. This process continues until the oral side is approximately equally exposed to the light in all positions on the spiral course. If the stentor is at *c* when *n* is exposed it responds at once and orients as described above. If the light from *n* is more intense than that from *m*, or if the organism is very sensitive when *n* is exposed and *m* shaded, it responds at once no matter in which position it is. If it is at *b* it turns toward the source of light, but now repeats the reaction, successively turning in various directions until it becomes oriented. (After Mast, 1911).

light. The orienting response therefore tends to keep it in light of moderate intensity, indicating that these responses are fundamentally adaptive.

IV. CILIATES

Very few of the ciliates respond to light, and only one of these, *Stentor coeruleus*, has been at all thoroughly investigated.

If the luminous intensity is rapidly increased, this organism stops, turns toward the aboral surface, and then proceeds. This is a shock-reaction, for if the intensity is slowly increased there is no response. If the intensity is decreased there is no response. If *Stentor* is exposed in a beam of light, it orients fairly precisely and swims from the light, i.e. it is photonegative. It rotates on the longitudinal axis as it swims, consequently when it is not oriented the oral and the aboral surfaces are alternately shaded and illuminated. The oral surface is much more sensitive than the aboral; therefore every time that this surface is carried from the shaded to the illuminated side, the result is the same as an increase in the illumination of the entire organism and it consequently responds, i.e. it turns toward the aboral surface. This continues until it is directed from the light, and rotation no longer produces changes of intensity on the two surfaces (Fig. 4). Photoc orientation in *Stentor* is therefore the result of a series of shock-reactions just as it is in *Euglena*. There is no evidence in support of the view that it is the result of a continuous quantitative difference in the activity of the cilia on opposite sides in proportion to the difference in the illumination of these sides. The process of orientation in this form is therefore not in accord with the Ray-Verworn theory (Jennings, 1904; Mast, 1906, 1911).

V. COLONIAL FORMS

The process of orientation is essentially the same in all the colonial forms in which it has been studied. It has, however, been more intensively investigated in *Volvox globator* than in any of the other species.

Volvox is a slightly elongated, globular colonial organism somewhat less than 1 mm. in diameter. It consists of numerous cells (zooids) each of which contains two flagella and an eyespot. The zooids are arranged in a single layer at the surface of the colonies. The eyespot in each zooid is directed toward the posterior end of the colony, but those at the anterior end are much larger than the rest (Fig. 5).

Mast (1927) presented evidence which demonstrates that the eyespots consist of a pigmented portion which is cup-shaped, a lens-like structure near the opening of the cup and photosensitive substance between this and the inner surface of the cup, and that the lens-like structure brings the longer incident waves of light to focus in the wall of the cup and the shorter in the photosensitive substance, after reflexion from the inner surface of the cup (Fig. 6).

Mast (1907, 1926 a, 1927 a, 1932 a) made a very thorough study of movement, response and orientation in *Volvox globator*. The more important of the results obtained in this study lead to the following conclusions:

The colonies rotate on the longitudinal axis as they swim. This is due to the diagonal stroke of the flagella. In a beam of light they usually orient and go fairly

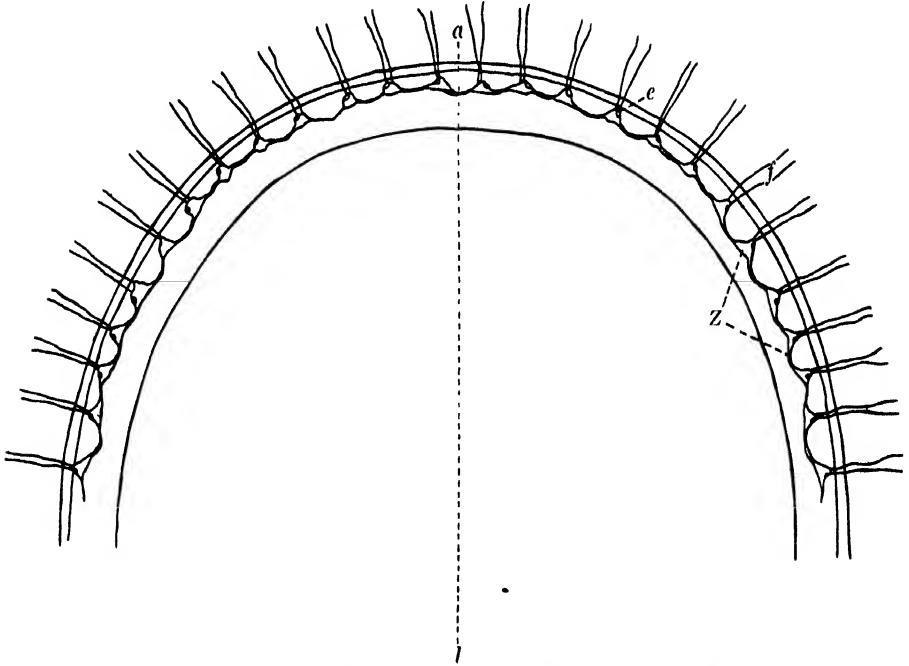


Fig. 5. Camera drawing showing the zooids in about one-half of an optical section through the longitudinal axis of a colony of *Volvox*. *l-a*, longitudinal axis of colony; *a*, anterior end; *Z*, zooids; *f*, flagella; *e*, eyes. Note that the eyes are located at the outer posterior border of the zooids and that they become larger as the anterior end of the colony is approached. (After Mast, 1926.)

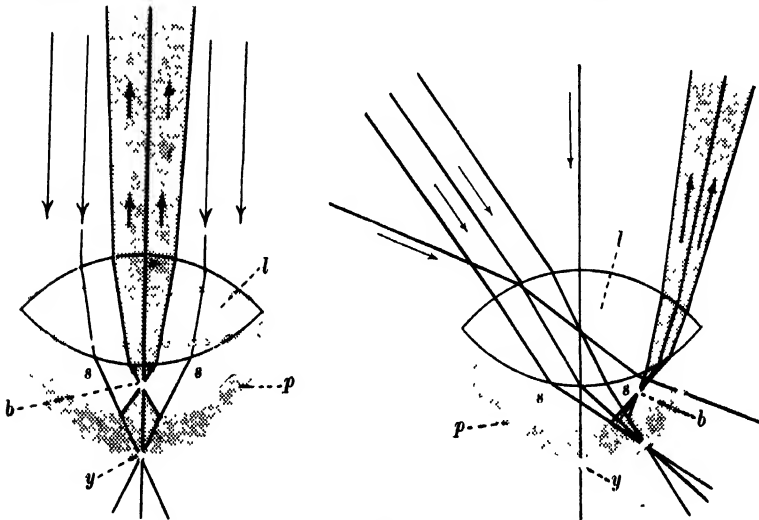


Fig. 6. Sketches showing the structure of the eyespot in *Volvox* and its action on light entering the pigment-cup at different angles. *p*, pigment-cup; *l*, lens; *y*, yellow focal spot; *b*, bluish green focal spot; *ss*, photosensitive substance; large arrows, incident rays of light. Note that the longer waves of the incident light are brought to focus in the wall of the pigment-cup and that the shorter waves are brought to focus in the cup, after being reflected from the inner surface, and then continue in the form of a concentrated beam of bluish green light. Note also that the more obliquely the incident light enters the pigment-cup the nearer the edge of the cup the yellow focal spot is located. (After Mast, 1927.)

directly either toward or from the light, i.e. they may be either photopositive or photonegative or neutral.

If the colonies are swimming toward the light and the intensity is rapidly decreased without any change in the direction of the rays, rotation on the longitudinal axis stops and forward movement increases greatly, but this continues for only a few seconds. If the intensity is increased, forward movement stops and rotation increases. If they are swimming from the light the reverse occurs, i.e. forward movement decreases if the intensity is increased, and increases if it is decreased. If the colonies are neutral, there are no such responses to changes of intensity. These responses consist chiefly, if not entirely, of rapid changes in the direction of the stroke of the flagella. In photopositive colonies rapid decrease in illumination causes the stroke to change from diagonally backward to straight backward and increase in illumination causes it to change from diagonally backward to sidewise. In photonegative colonies precisely the reverse obtains. If the luminous intensity is slowly changed these responses do not occur. They are therefore dependent upon the rate of change of intensity, i.e. they are shock-reactions which are somewhat similar to those observed in *Euglena*.

If *Volvox* is kept in low illumination or in darkness for several hours it becomes inactive, and if the illumination is now increased it gradually becomes active again. These responses consist chiefly, if not entirely, in changes in the rate or the efficiency of the stroke, not in changes in the direction of the stroke of the flagella. They are relatively slow responses which occur even if the luminous intensity is gradually changed. They are primarily dependent upon change in luminous intensity, not upon the rate of change. There are consequently in *Volvox* two different types of response, typical shock-reactions and responses which are often called kinetic responses.

If a colony of *Volvox* in a beam of light is laterally illuminated, it turns gradually until it is oriented and then proceeds either toward or from the light. When it is laterally illuminated, the zooids, owing to rotation of the colony on the longitudinal axis, are continuously transferred from the light side to the dark side and vice versa. As the zooids pass from the light side to the dark side, the photosensitive substance in the eyespots becomes shaded by the pigment cup and as they pass from the dark side to the light side this substance becomes fully exposed. The rapid decrease in the illumination of the sensitive substance on the dark side induces shock-reactions on this side which, in photopositive colonies, consist of increase in the backward phase of the stroke of the flagella; and the rapid increase in the illumination of this substance on the light side induces shock-reactions on this side which consist of increase in the lateral phase of the stroke of the flagella (Fig. 7). This difference in the direction of the stroke of the flagella causes the colonies to turn gradually toward the light until they are directed toward it, after which all sides are equally illuminated, rotation on the longitudinal axis no longer produces changes in the illumination of the photosensitive substance, and the shock-reactions cease. They continue to proceed directly toward the light because, in the absence of external stimulation, they tend to take a straight course and because, if they are forced out of their course,

opposite sides immediately become unequally illuminated, resulting in change in the intensity of the illumination of the photosensitive substance in the eyespots and consequently in reorientation.

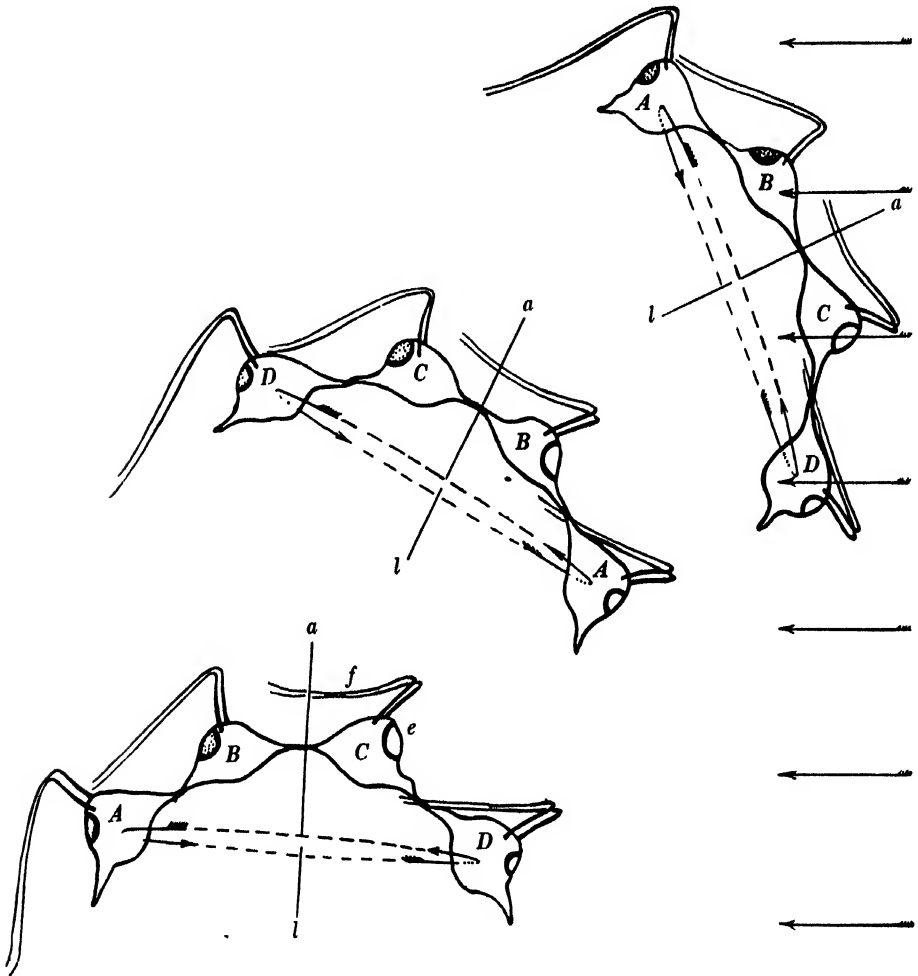


Fig. 7. Diagrammatic representation of the process of orientation in *Volvox*. A, B, C, D, four zooids at the anterior end of the colony; *l*-*a*, longitudinal axis; large arrows, direction of illumination; small arrows, direction of locomotion; curved arrows, direction of rotation; *f*, flagella; *e*, eyes, containing a pigment-cup represented by a heavy black line and photosensitive tissue in the concavity of the cup. Note that when the colony is laterally illuminated the photosensitive tissue in the eyes on the side facing the light is fully exposed and the flagella on this side beat directly backward, while that in those on the opposite side is shaded by the pigment cup and the flagella on this side beat directly backward. The difference in the direction of the beat of the flagella on these two sides is due to alternate decrease and increase in the luminous intensity to which the photo-sensitive tissue in the eyes is exposed, owing to the rotation of the colony on its longitudinal axis, an increase causing, in photopositive colonies, a change in the direction of the stroke of the flagella from backward or diagonal to lateral, and a decrease a change from lateral or diagonal to backward. In photonegative colonies precisely the opposite obtains. In photopositive colonies this results in turning toward and in photonegative colonies turning from the source of light. In both the turning continues until opposite sides are equally illuminated, when changes of intensity on the photosensitive tissue are no longer produced by rotation and the orienting stimulus ceases. (After Mast, 1926.)

If colonies are exposed in a field of light consisting of two horizontal beams which cross at right angles, they orient and swim toward a point between the two beams. The location of this point depends upon the relative intensity of the light in the beams. The higher the intensity in one of the beams in relation to that in the other, the nearer to the former the point is. If the intensity in the two beams is equal the point is half-way between them, and when the colonies are oriented under these conditions, opposite sides are equally illuminated, both in reference to intensity and direction of the rays, i.e. the angle of incidence at the surface of the colony. But if the intensity in the two beams is not equal the illumination of the oriented colonies is higher and the angle of incidence is greater on one side than on the other. However, when a colony is oriented in a field of light, no matter how unequal the intensity from different directions may be, transfer of the zooids from side to side, owing to rotation on the longitudinal axis, causes no responses, i.e. the effect of unequal illumination on opposite sides is equal. This obviously must be correlated with the difference in the angle of incidence. Mast & Johnson (1932) demonstrated that the location of the point of focus in the eyespot varies with the angle of incidence and that the photosensitive substance in the eyespot is more sensitive in the central regions of the eyespot than at the periphery. The stimulating efficiency of light, therefore, depends upon the location of the point of focus which in turn depends upon the angle of incidence. The equal effect of light on opposite sides of colonies which are unequally illuminated on opposite sides when they are oriented, is therefore due to the fact that the point of focus in the eyespots is more nearly centrally located on the side which receives least light than on that which receives most.

In negative colonies the process of orientation is precisely the same as it is in positive colonies, except that decrease in intensity causes increase in the lateral phase, and increase in intensity increase in the backward phase of the stroke of the flagella, resulting in more rapid movement of the illuminated than of the shaded side of the colonies and consequently in turning from the light in place of toward it.

Orientation of *Volvox* in light is consequently the result of qualitative difference in the action of the locomotor appendages on opposite sides, due to shock-reactions induced by rapid change in the intensity or the location of the light in the photosensitive substance in the eyespot, caused by rotation of the colonies on the longitudinal axis, *not the result of quantitative difference*, due to continuous action of the light. It is therefore not in accord with the Ray-Verworn theory.

VI. WORMS

(1) *Larvae of Arenicola*

The larvae of most of the marine worms are intensely photopositive when they leave the eggs. Owing to this they come to the surface of the water and scatter. Later they become photonegative and go to the bottom where they burrow in the mud. The process of orientation has, however, not been thoroughly investigated in any of these larvae except those of *Arenicola*. The larvae of *Arenicola* are finger-like in form and about 0.3 mm. long. They have two eyes and a band of cilia near either

end. They swim for a time after they hatch, then settle to the bottom and crawl. They orient precisely and are strongly positive when they swim and negative when they crawl.

Swimming is the result of ciliary action, but orientation is the result of muscular contraction. The larvae rotate on the longitudinal axis when they swim, so that when they are laterally illuminated, each eye is alternately directed toward and away from the light, resulting in alternate increase and decrease in the illumination of it. When, in the process of rotation, either of the eyes comes to be directed toward the light, the muscles on the illuminated side of the larva contract violently, and this turns the head toward the light. This occurs twice during each rotation and soon results in orientation, after which, if the light is from a single source, the two eyes are continuously equally illuminated and muscular contraction ceases (Fig. 8).

Mast (1911) maintains that these muscular contractions are reflexes dependent upon the rate of change of luminous intensity in the eyes, and that the orienting stimulus consequently ceases after the organism is oriented. Garrey (1918) holds that they are the result of difference in tonus in the muscles on opposite sides, due to difference in the amount of light received by the two eyes; that the tonus of the muscles on either side is continuously proportional to the amount of light received by the eye on that or the opposite side; and that the orienting stimulus consequently continues after the organism is oriented, being equal when the two eyes are equally illuminated, i.e. when the organisms swim directly toward the light.

Mast demonstrated that if the larvae are held so that they cannot rotate, and the light in one eye is increased or that in the other is decreased, the muscle on the more highly illuminated side contracts, but it is unfortunately not known how long this position is held. The results in hand consequently show that contraction of the muscles is correlated with difference in the intensity of the illumination of the two eyes, but they do not show whether it is due to difference in tonus in accord with Garrey's contention or change of intensity in accord with Mast's contention. It may, however, be said that the response occurs so rapidly that there does not appear to be time to induce change in what is ordinarily called tonus.

There is no evidence concerning localization of the stimulus in the eye. The fact, however, that locomotion is due to ciliary action and orientation to muscular action makes it evident that the process of orientation is not in accord with the Ray-Verworn theory.

(2) *Earthworms*

The earthworms respond very definitely to light and some of them orient fairly precisely. The process has been thoroughly studied only in *Lumbricus terrestris* and *Eisenia foetida*.

Hesse (1896) and Hess (1925) demonstrated that there are in the epidermis and in enlargements near the epidermis of some of the nerve cords large cells, and that each of these cells contains a highly refractive body surrounded by a nerve-net. They found that the distribution of these cells is essentially the same as the distribution of sensitivity to light and they concluded that they are photoreceptors.

If the light in which specimens of these worms have become quiet is rapidly and greatly increased they raise the anterior end and swing it violently from side to side in an exploratory fashion. If the light is increased slowly they do not raise the

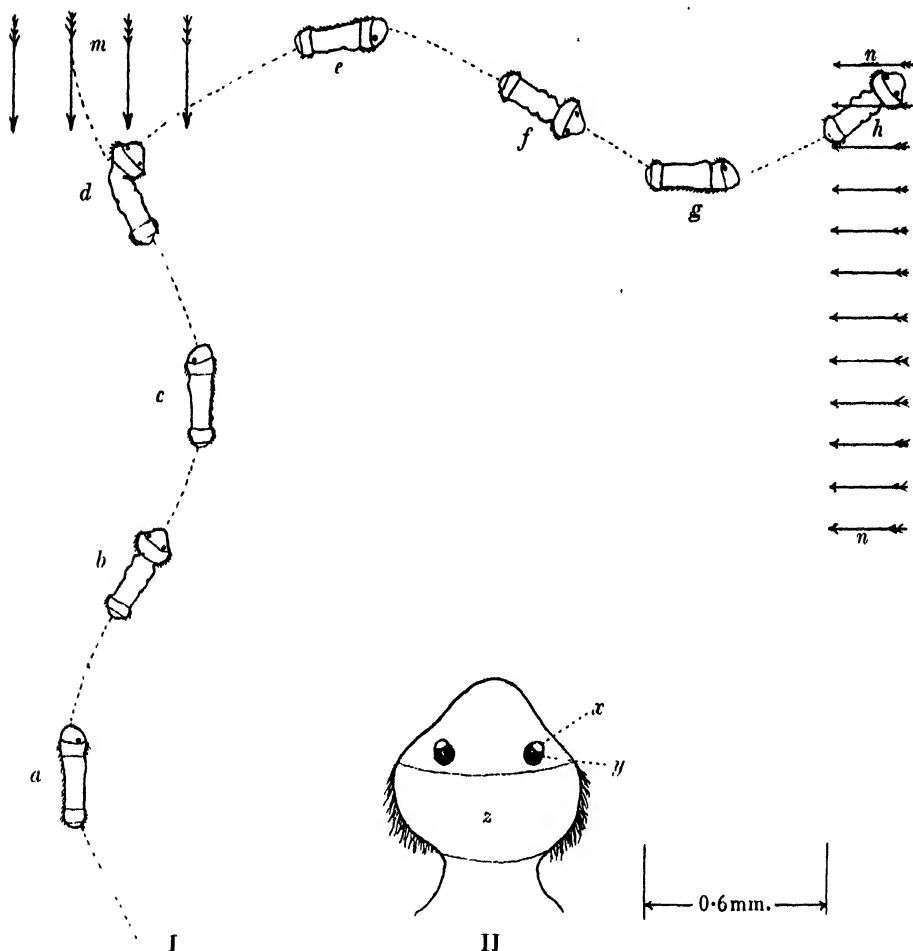


Fig. 8. I. *Avenicola* larva in the free-swimming state, proceeding on a spiral course. *m*, *n*, directions of light; *a*–*h*, different positions on the spiral; *b*, dorsal surface up, right eyespot toward *n*; *d*, ventral surface up, left eyespot toward *n*. If the ray direction is changed by simultaneously exposing *n* and shading *m* when the larva is in position *a* or *c*, no reaction takes place until it reaches *b* or *d*, then it bends the head sharply toward the source of light and turns in its course. In the former position it turns toward the right side of the body, in the latter toward the left. II. Sketch showing the general structure and position of the eyespots as seen under the oil immersion objective. *y*, dark brownish cap-like portion of eye; *x*, colourless portion of eye; *z*, band of cilia. (After Mast, 1911.)

anterior end but they gradually become active. The former response is therefore closely correlated with the rate of change in intensity, and the latter is not.

Parker & Arkin (1901), Smith (1902) and Adams (1903) maintain that if earthworms are illuminated from one side the anterior end usually turns directly from the light and continues until they are oriented. Holmes (1905) however is of the opinion that the worms start to turn toward the light as often as from it but that movement

toward the light is only slight, owing to inhibition caused by exposure of the highly sensitive tip. He believes Parker & Arkin and others overlooked slight preliminary movements which occur before the actual extension takes place and that this accounts for the preponderance of negative turning recorded. He concludes that orientation is brought about by random movements and retention of positions in which the anterior end is least exposed to light. Harper (1905) comes to the same conclusion in reference to orientation in weak light but he maintains that in strong light orientation is direct and random movements are practically eliminated.

Mast (1911) found that the earthworm when it is crawling usually swings the anterior end alternately from one side to the other; and that if it is laterally illuminated while it is progressing thus, it responds either by contraction or extension of the anterior end or by suddenly raising this end and swinging it from side to side or by turning directly either toward or from the light, and that the character of the response depends upon the state of adaptation and the activity of the worm and the increase in the intensity of the light to which it is subjected.

The results obtained in numerous detailed observations lead to the following conclusions:

If the worm is active the alternate turning to the side in ordinary locomotion is greater than if it is sluggish, and if while it is in this condition, it is illuminated from one side, the anterior end turns sharply in the direction opposite that toward which the anterior is pointing when it is illuminated. Under these conditions it turns toward the light practically as often as from it, regardless of the extent of increase in intensity. If it is sluggish and side to side movement of the anterior end is slight, it turns directly from the source of light with very few exceptions. If it is sluggish and at rest and the lateral illumination is not very intense the worm does not respond until a few moments after the exposure, then it very slowly extends and simultaneously turns the anterior end from the source of light without exception. Under such conditions the movements are so slow that they can readily be followed in detail under a hand lens. No evidence was obtained indicating even the slightest preliminary turning toward the source of light in any of the specimens observed. It is consequently obvious that while random movements and retention of positions in which the anterior end is to some extent shaded doubtless play a prominent part in orientation under some conditions, they are not involved under others. Orientation under some conditions is therefore brought about by direct turning from the more highly illuminated side. Whether this turning is in the nature of differential response to localized stimulation or is due to difference in tonus of the muscles on opposite sides is not known. However, results obtained by Hess and Prosser throw some light on this problem.

Hess (1924) found that if the brain is removed, earthworms become positive to light of very much higher intensity than normally obtains. Prosser (1934) found that reduction in temperature, injection of depressant drugs around the brain and injury of certain parts of the brain have the same effect. Prosser reaches the following conclusions concerning the processes involved in these phenomena:

In specimens with the brain removed, lateral illumination produces impulses in

the photoreceptors on the illuminated side, which pass through the nerve cord to the muscles on this side and cause them to contract. This results in turning toward the light. In normal specimens, in addition to the impulses which pass through the cord to the muscles on the illuminated side, there are impulses which pass through the brain to the muscles on the opposite side. These come from the receptors in the highly sensitive anterior end of the worm. They are therefore much stronger than those which originate farther back and pass through the cord. The latter are consequently inhibited by the former; this results in contraction of the muscles on the shaded side and turning from the light.

The facts in hand appear to show then that orientation of earthworms in light is due to contraction of muscles on one side of the body and that this contraction is ordinarily controlled by impulses which originate in photoreceptors on the opposite side, i.e. that the region of contraction is directly correlated with the localization of the stimulus, but that the impulses are modified and integrated in the central nervous system. They also show that under some conditions contraction occurs in the muscles under greater tension regardless of the location of the stimulus and that this results in random movements which often play a very prominent role in orientation.

(3) *Turbellaria*

Locomotion in the turbellaria is produced by the action of cilia on the ventral surface, but the direction of locomotion is controlled by the action of muscles in the body as it is in the larvae of *Arenicola*. Some orient fairly precisely in directive illumination and change the rate of locomotion if the intensity is changed; others do not orient but change the rate of locomotion.¹ Some of those which orient are photonegative and some are photopositive.

If the intensity of the light is rapidly and extensively increased or decreased, they raise the anterior end and swing it from side to side; if it is slowly changed or if the eyes have been removed there is no such response. This response is therefore dependent upon the effect of the rate of change in luminous intensity on the eyes. It is due to contraction of muscles in the body. If normal specimens or specimens without eyes are kept in constant light they come to rest and if the intensity is now changed, they very gradually become active again. This response is, therefore, not dependent upon the eyes and is not closely correlated with the rate of change in intensity. It is due to the effect of the light on the action of the cilia and this effect is similar to the effect of temperature (Loeb, 1906; Walter, 1907; Mast, 1911).

Mast (1910 a) made observations on the process of orientation in four different species of marine turbellaria. He found that all are photonegative and orient fairly precisely, but that if the eyes are removed they no longer orient. This seems to indicate that orientation depends upon balanced action of the muscles on opposite sides, correlated quantitatively with the relative amount of light received by the eyes on opposite sides in accord with the Ray-Verworn theory. He found, however,

¹ Results recently obtained by Ulliyott (1936) in observations on *Dendrocoelum lacteum* throw considerable doubt on the validity of this statement. He says (p. 267): "No differences in the rate of movement could be detected over a range of light intensities from 0 to 2500 ergs/cm.²/sec."

that specimens with one eye removed, either by gouging it out or by cutting it off, orient after recovery from the operation nearly as precisely as normal specimens and that in the process of orientation they turn from the light no matter whether it is directed toward the blind side or the normal side, but he did not ascertain the details involved in the process of turning. This was done by Taliaferro, as will be shown presently.

The fact that specimens with only one eye orient, shows that the process involved is not necessarily dependent upon balanced action in the two eyes and that the action of the muscles on both sides can be controlled by impulses from either side.

Kühn (1919, p. 12) in considering the results obtained by Pearl (1903), Boring (1912) and Steinmann & Bresslau (1913) in observations on various planaria comes to the following conclusion: "In den Sinneszellen der Augen wird der physikalische Reiz in den physiologischen Vorgang der Erregung umgewandelt, und diese wird durch den nervösen 'Reflexbogen' vorwiegend nach der Längsmuskulatur der gegenüberliegenden Körperseite geleitet. Wird das Tier von einer Seite belichtet, so wird das Auge dieser Seite stärker erregt und der Muskulatur der Gegenseite eine stärkere Erregung zugeführt, die eine stärkere Dauerverkürzung, 'tonische' Verkürzung, der Muskulatur bewirkt." He consequently holds that the process of orientation in planaria is in full accord with the Ray-Verworn theory as elaborated by Loeb, Bohn, and others.

Taliaferro (1920) made a very intensive study of the process of orientation in *Planaria maculata* under very accurately controlled conditions and found the following:

If *Planaria maculata* is illuminated from one side with light of moderate intensity, the opposite side contracts, resulting in gradual turning until it is oriented, after which it proceeds fairly directly from the light. If the intensity is higher, it turns more rapidly, and if it is very high, it may first swing the anterior end from side to side several times and then turn from the light. It therefore orients directly under some conditions and indirectly under others, depending upon the intensity of the light.

If the two eyes are removed, either by careful dissection or by cutting the head off, it does not orient, but if the intensity of the light is changed its activity changes. Orientation, which is controlled by muscular action, is therefore dependent upon stimuli received through the eyes and activity which is controlled by ciliary action is not so, or at any rate not entirely.

If one eye is dissected out with as little injury to the surrounding tissues as possible the wound heals in 24 hours or less; then the animal appears normal in every respect except that it has but one eye. Taliaferro says (p. 88): "When observed in non-directive light, specimens with one eye removed travel about, apparently, in every respect like normal individuals. In neither directive nor non-directive light is there any evidence of sluggishness, circus movements, or other abnormal motor activities." By cutting out a portion of the remaining eye in such specimens and illuminating the animal from various directions, Taliaferro proved that the eye is stimulated only by those rays which enter the rhabdomes parallel or nearly parallel

with the longitudinal axis, and by accurately directing the light into different regions of an intact eye he demonstrated that if the rhabdomes at the anterior end or in the central portion of the eye are stimulated the animal turns from the side stimulated, but that if those at the posterior end or the ventral edge of the eye are stimulated it turns toward the side stimulated (Fig. 9). It was thus demonstrated for the first time that the nature of the response in animals with relatively simple eyes depends upon the location of the stimulus in the eye, a fact of fundamental importance in the analysis of behaviour, as clearly indicated by the results obtained in the study of the process of orientation in specimens with only one eye.

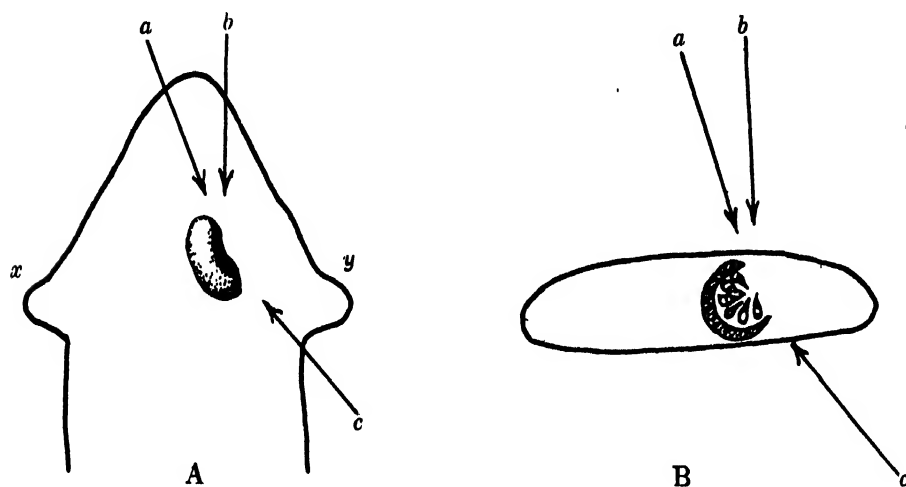


Fig. 9. Diagram representing the direction of turning when a specimen of *Planaria* with left eye removed is illuminated from different directions. A, dorsal view; B, cross section at $x-y$. A. If the light enters the eye from any point between the arrows $a-b$, the specimen turns toward the normal side. If it enters the eye from any point between the arrows $b-c$, the specimen turns away from the normal side. If the eye is illuminated from any point between $a-x$ or $c-x$ there is no response. (Modified after Taliaferro, 1920.)

If such specimens are illuminated from the normal side, they turn directly from the light until they are oriented and then continue fairly directly from the light. If they are illuminated from the blind side, they continue without any response until, owing to random wandering or swinging or twisting of the anterior end, the light enters the intact eye (Fig. 10). If it enters the posterior or the ventral rhabdomes in the eye, they turn toward the normal side, i.e. from the light, and soon become oriented. If it enters the anterior or the dorsal rhabdomes, they turn from the normal side, i.e. toward the light. This turning may continue until they are directed from the light, or there may be a series of successive responses which finally result in orientation. The direction of turning, i.e. the nature of the orienting response, therefore, depends upon the location of the stimulus in the eye as well as upon the intensity of the light. These responses are rapid and of short duration. They are probably dependent upon rate of change in intensity, i.e. they are probably shock-reactions. Orientation in one-eyed specimens is therefore due to one or more re-

sponses, consisting of rapid muscular contraction on one side. It obviously is not due to "balanced" stimulation of photoreceptors on opposite sides, resulting in "balanced" tonus of muscles on opposite sides.

In normal specimens the process of orientation is doubtless fundamentally the same as it is in specimens with but one eye. It is obvious, however, that the effect of illumination and change in illumination in one eye is modified or inhibited by the effect of simultaneous illumination and change in illumination in the other. This matter will be more fully considered presently.

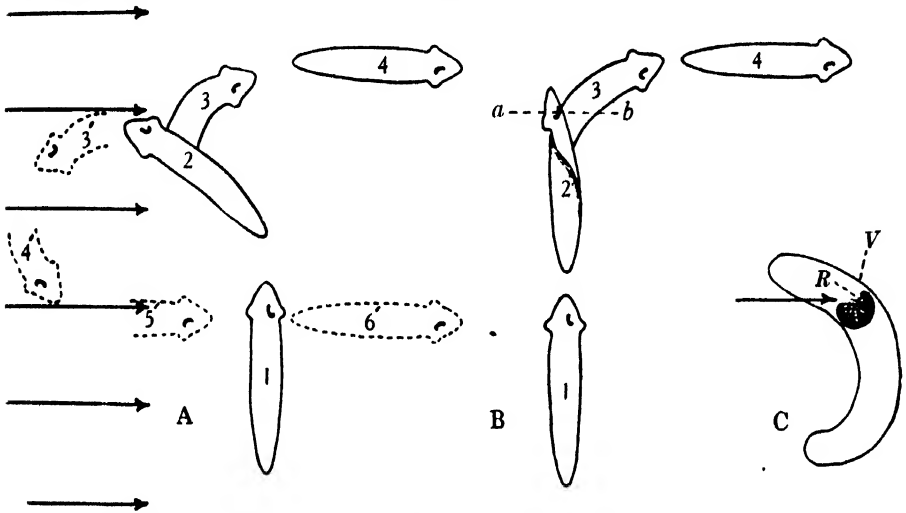


Fig. 10. Diagram illustrating process of orientation in *Planaria* with left eye removed, when illuminated laterally from the left, i.e. the "blind" side. Arrows, direction of light; A, orientation by means of "wandering reflex"; 1, 2, 3, 4, successive positions of animal; 1, 2, 3', 4', 5' and 6', successive positions of the animal if it is moving rapidly; B, orientation by means of "twisting reflex"; 1-4, successive positions of specimen; C, diagrammatic cross-section of specimen B through the plane $a-b$; R, rhabdomes; V, ventral surface. Note that when the end is twisted (B, 2) the rhabdomes in the ventral portion of the eye are illuminated and that this causes the worm to straighten and then turn toward the normal side (B, 3). (Modified after Taliaferro, 1920.)

VII. INSECTS

(1) *Flies, beetles, bees, butterflies and moths*

Orientation in light is very common in insects. Some go toward or from the source of highest illumination, others tend to go toward definite luminous points in space. In light from two sources, the former usually go toward or from a point between the two sources, the latter usually directly toward one of the two sources.

If a photopositive specimen is laterally illuminated by light from a single source so that one eye receives more light than the other, it turns until both eyes are approximately equally illuminated and then proceeds toward the light, often remarkably directly, but if one eye is covered with opaque substance it deflects toward the functional eye and tends to take a circular course.

These facts led Loeb (1906), Bohn (1909), Garrey (1918) and others to conclude that the tonus of the muscles in the legs of insects is proportional to the amount of

light received by the eye connected with the muscles, and that this results in movement of the legs on either side proportional to the amount of light received by the eye on the opposite side, and consequently in turning when the two eyes are unequally illuminated and in direct movement when they are equally illuminated, i.e. that orientation in insects is in principle in accord with the Ray-Verworn theory. There is a number of well-established facts which indicate that these views are not valid.

(1) Rádl (1903) long ago observed that the blow-fly, *Calliphora vomitoria*, orients nearly as precisely with one eye covered as it does with both eyes functional. Holmes (1905 a) discovered that, while *Ranatra* with one eye covered at first de-

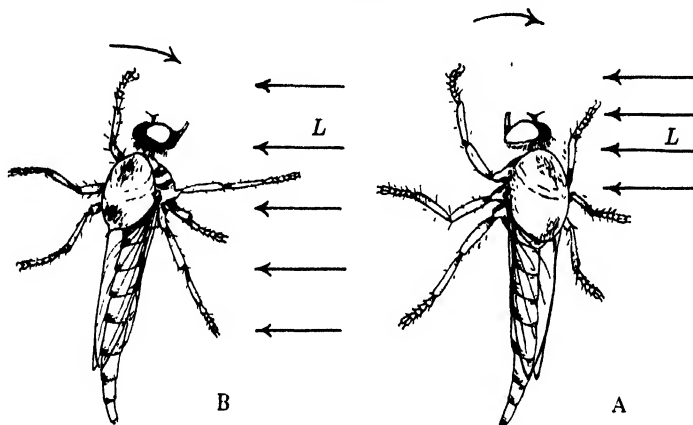


Fig. 11. *Proctacanthus* on a black background in a well-defined horizontal beam of light, *L*. A, right eye covered. Note that it is leaning toward the covered eye and that the upper surface of the functional eye is directed toward the light. B, *Proctacanthus* on a white background in front of a window; the upper portion of the left eye and the lower portion of the right eye covered. Note that it is leaning to the left and turning to the right toward the light, *L*. (After Mast, 1924.)

flects strongly toward the functional eye and makes definite circus movements, the deflexion gradually decreases until it has practically disappeared and that then the specimens orient and go toward the light nearly as precisely as normal specimens. Similar results were obtained in all other insects in which the response to light under these conditions has been thoroughly studied, namely, *Drosophila* (Carpenter, 1908), *Vanessa* (Dolley, 1916), *Apis* (Minnich, 1919), *Eristalis* (Mast, 1923) and others. It is obvious that orientation in these insects is not necessarily correlated with the relative amount of light received by the two eyes.

(2) In the robber-fly, *Erax rufibarbis*, with one eye covered, the body assumes a tilted posture. The legs on the blind side are much more extended than those on the normal side and the feet consequently tend to move faster on the former than on the latter side, resulting in turning and circus movements toward the normal side (Garrey, 1918). Similar reactions are obtained if the lower half of one eye, the right, for example, and the upper half of the other eye are covered, i.e. the insect leans toward the right side and turns to the right; but if such a specimen is laterally illuminated from the left, it turns, under certain circumstances, to the left (Fig. 11), i.e. toward the side on which the extension of the legs is greater, in a direction pre-

cisely opposite to that which would obtain if turning were due to difference in the extension of the legs on opposite sides, in accord with the tonus hypothesis (Mast, 1924).

(3) If a photopositive insect in a field of light consisting of two beams crossing at right angles goes toward a point between the two beams the location of the point depends upon the difference in the intensity of the beams. The greater the difference the nearer the more intense beam the point is located (Dolley, 1916; Mast, 1923). Results obtained in observations made on *Eristalis tenax* by Dolley & Wierda (1929) show that with the intensity of the two beams so related that when the insect is oriented the image in one eye is near the anterior end and that in the other eye is near the posterior end, the one receives fifty times as much light as the other. That is, these results show that to produce a given effect on turning, an image near the anterior end of the eye must be fifty times as intense as an image of the same size near the posterior end. This indicates that the efficiency of light to induce turning decreases rapidly as the location of the image passes forward in the eye. In an oriented insect under these conditions the two eyes are equally illuminated only when the light in the two beams is equal (Mast, 1923). Moreover, Parker (1903) and Cole (1907) demonstrated that a given amount of light in a large image has a greater effect on turning than the same amount of light in a small image. It is therefore evident that when an insect is oriented under natural conditions, the two eyes are rarely if ever equally illuminated.

(4) Dolley (1916) demonstrated that *Vanessa* in a beam of light orients with the longitudinal axis at an angle with the direction of the rays and goes diagonally across the beam, that the magnitude of this angle is independent of the light intensity; and that if the intensity is rapidly increased or decreased after the insect is oriented, it turns sharply from or toward the light and then gradually back until it has assumed its former direction of movement, but that if the intensity is slowly changed, it does not turn. These responses are therefore typical shock-reactions. Continuous action of light is not involved.

(5) If photopositive insects on a non-reflecting background are (with a concentrated source of light) illuminated on one side from behind, all the legs on the illuminated side step backward and all on the opposite side forward (Fig. 12). If illuminated directly from the right, the front legs on both sides step toward the right. If illuminated directly from the front, all the legs on both sides step forward. This obtains also for insects with only one functional eye. The movement of the legs on both sides is controlled by photic stimulation of either eye and the direction of movement depends upon the location of the stimulus in the eye (Mast, 1923, 1924).

(6) Photopositive insects with the front and the middle legs on one side removed orient nearly as precisely as normal insects. If they are laterally illuminated from either direction, the front leg is extended toward the light, attached and then flexed (Figs. 13 and 14). Thus the anterior end is pulled either toward the injured or toward the normal side, depending upon which side is more highly illuminated. After they are oriented, the front leg is extended nearly directly forward, attached and then flexed (Fig. 13). Thus they proceed slightly sidewise but fairly directly toward the

light, with the two eyes continuously unequally illuminated and the action of locomotor appendages on opposite sides not balanced (Mast, 1923).

(7) *Eristalis* on the wing orients fairly accurately. If, after it is oriented in a beam of light, the source of light is raised or lowered, it turns directly upward or downward. When the position of the light is changed, the images in the two eyes are equally and simultaneously changed in location. The turning is due to this change. It cannot be due to difference in the illumination of the two eyes, for they are continuously equally illuminated, and it evidently cannot be due to difference in the action of the locomotor appendages on opposite sides (Mast, 1923). Similar results

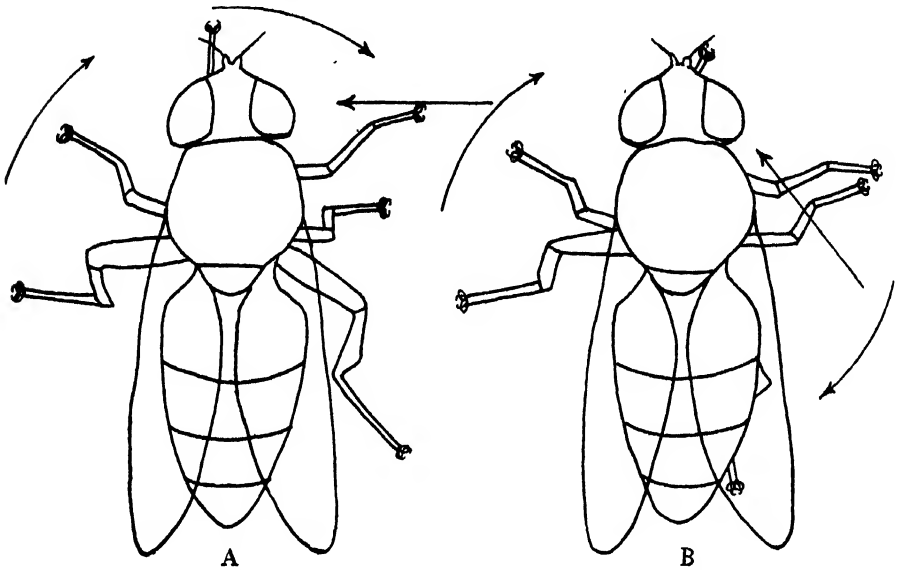


Fig. 12. Sketches of *Eristalis* showing the direction of movement of the feet during the process of orientation in a horizontal beam of light. A, rays perpendicular to the longitudinal axis; B, rays directed toward the posterior surface of one eye; straight arrows, direction of the rays; curved arrows, direction of movement of the feet. Note that both front feet move sidewise toward the light and that the farther back in the eye the region toward which the rays are directed is, the more marked this sidewise movement is. Note also that when the rays are directed toward the posterior surface of the eye all the feet on one side move forward while all those on the other move backward. (After Mast, 1923.)

were obtained in observations on *Caprella* (Mast, 1911), and similar responses doubtless occur in all photopositive or photonegative animals which fly or swim and do not rotate on the longitudinal axis as they progress.

(8) If a male fire-fly is some distance from a female fire-fly when she produces a flash of light, he, no matter in which direction he may be going, turns until he faces the region from which the light came and then proceeds. Thus after momentary illumination, he, in total darkness, turns in any direction through an angle between 0 and 180°, depending upon the direction in which he faced in relation to the location of the female when she produced the flash of light. If a male fire-fly glows in the neighbourhood of a female, she raises and twists the abdomen until the ventral surface is directed toward him and then glows, no matter where he is located. Thus,

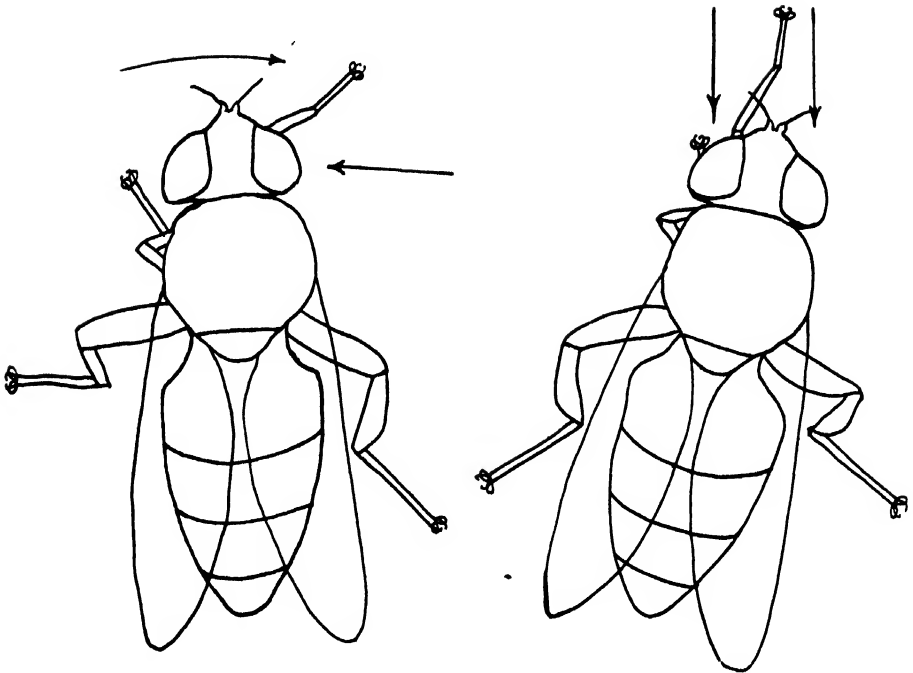


Fig. 13. Sketches showing the direction of movement of the feet in *Eristalis* with the right front and middle legs removed. Arrows, direction of rays of light. Note that when the insect is illuminated from the right the front leg is well extended toward the right. In this position the foot is attached to the substratum after which the leg is flexed and the anterior end of the insect pulled to the right. Turning to the left is accomplished in the same way except that the front leg is extended to the left. The other legs assist in these movements. Note also that when the insect is illuminated from in front it goes toward the light somewhat sidewise and that the front is extended nearly directly forward. When it is in this position the foot is attached to the substratum, then the leg is flexed and the insect pulled forward. (After Mast, 1923.)

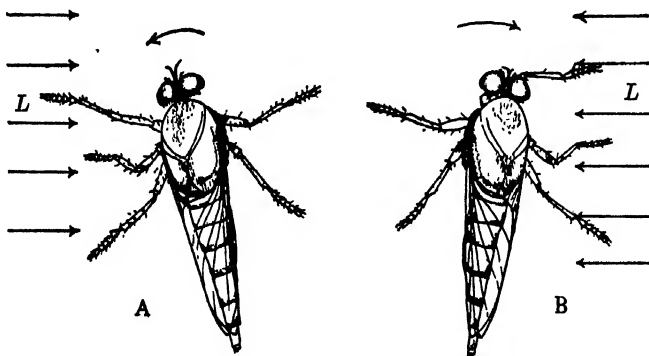


Fig. 14. *Proctacanthus* with one front leg removed. A, turning to the left toward the light; B, turning to the right toward the light; L, beam of light. Note the positions of the front leg. It is used in pulling the animal to the right as well as to the left. (After Mast, 1924.)

after an image of a flash of light produced by a male has been formed on the retina of a female, she, in total darkness, directs the ventral surface of the abdomen toward any point in space, depending upon where the male chances to be when he glows, and these responses, both in the male and the female, often occur when only one eye is illuminated. It is therefore obvious that they cannot be accounted for by any theory that demands continuous action of the light and balanced illumination of the receptors on opposite sides in the process of orientation (Mast, 1912; Buck, 1937).

(9) Many insects, after having assumed a definite axial position in relation to a source of light, tend to retain this position. This phenomenon is particularly prominent in a complex field of light, e.g. in direct sunlight in an open field. If these insects are rotated on their sagittal axis (on a turn-table, for example), they turn in the opposite direction. They apparently tend to take such a course that the location of the images in the eyes remains constant. This has been observed in the beetle *Coccinella* by Rádl (1903); in ants by Santschi (1911); in bees by Wolf (1926, 1931); and in a considerable number of other insects by Buddenbrock (1917, 1919, 1931 a), Buddenbrock & Schulz (1933), Fraenkel (1927), Willrich (1931), Schulz (1931), and Niemczyk (1923).

These are pertinent facts in reference to the factors involved in the process of orientation of insects. How can they be explained?

If in *Eristalis* with one eye covered, the stimulus is located at the posterior edge of the retina, the feet on one side move forward while those on the other side move backward, the two front feet deflecting toward the side stimulated, the two hind feet from this side. If it is located in the lateral portion, both front feet move laterally toward the light, as do also the middle feet but to a less extent. If it is located in the central part of the anterior surface of the eye, the feet on both sides move forward and the insect does not turn. If it is located at the antero-median edge, it turns toward the covered eye.

These facts demonstrate that stimulation of any given region in the retina in either eye induces co-ordinated reactions in all the legs on both sides and that the character of these co-ordinated reactions is specifically correlated with the location of the stimulus in the eye. They also demonstrate that stimulation of any given region back of the centre of the anterior surface of the eye, induces reactions of such a character as to turn the insect toward the eye. The results obtained in other observations show that if the illumination is momentary, *Eristalis* turns through only a very small angle, but that if the illumination is continuous it turns until it faces the light, and that the continuous turning is due to the facts that the image of the source of light on the retina travels forward, stimulating successively different points and that each point stimulated causes further turning until the image reaches the anterior portion of the eye. This turning is therefore brought about by a series of reflexes, each specifically correlated with the location of the stimulus in the eye (differential responses to localized stimulation). These reflexes are similar to the scratch reflexes in higher forms induced by stimulation of various points on the surface of the body. And the character of each is dependent upon the location of the stimulus in the eye just as the character of each scratch reflex is dependent upon the localization of the

stimulus on the surface of the body. If both eyes are simultaneously stimulated other processes are involved as is indicated in the following paragraphs.

If *Eristalis* is exposed to light from two sources at the same distance and equal in size and intensity and so arranged that the rays cross approximately at right angles at the place of exposure, it turns until it faces a point half-way between the two sources and then proceeds toward this point. If the two sources of light differ in intensity it will turn until it faces a point nearer the more intense source and the greater the difference the nearer this source the point is. Under the first conditions

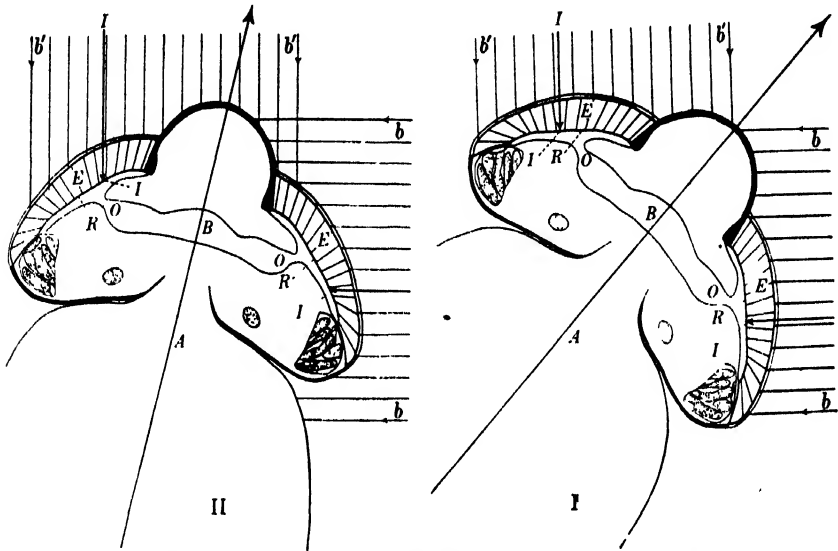


Fig. 15. Camera outlines of horizontal sections of the head of *Eristalis* showing the location of stimuli in specimens oriented in light from two sources. *A*, the two sources of light equal in size, intensity and distance; *B*, the two sources of light equal in size and distance, but unequal in intensity; *b-b* and *b'-b'*, horizontal beams of light from two lamps so far removed that the rays are practically parallel; *A*, longitudinal axis of the insect; *E*, eye with lines showing accurately the direction of the longitudinal axis of the ommatidia in different regions; *B*, brain; *O*, optic nerve; *R*, retina; *I*, image of lamp on the retina. Note that if an insect is oriented in light from two sources of equal size and intensity and at equal distances, *I*, the two eyes are equally illuminated, there being one image in either eye, both located in the same relative position and equal in size and luminous intensity; but that if it is oriented in light from two sources of unequal intensity, *II*, the two eyes are not equally illuminated, the image of the stronger source of light being farther forward than that of the weaker (After Mast, 1923.)

the two eyes are equally illuminated when the insect is oriented; under the other conditions they are not; i.e. the region stimulated is more intensely illuminated and farther forward in one eye than it is in the other; and the greater the difference in the intensity of the two sources, the greater the difference in the location and in the intensity of the illumination of the regions stimulated in the two eyes (Fig. 15). Consequently the turning effect of stimulation in a given region of the retina of one eye is obliterated by simultaneous stimulation of the same region in the opposite eye, provided the stimuli are of the same magnitude, and by simultaneous stimulation of any other region in the retina of the opposite eye provided the stimuli in the two eyes bear the proper relation in magnitude. If the stimulus in one eye is located

relatively farther forward than that in the other eye, the former in order to produce complete inhibition must be stronger than the latter, if farther backward it must be weaker.

The elimination of the effect of stimulation in one eye by simultaneous stimulation in the other eye is not due to antagonistic action of the legs on opposite sides as demanded by the Ray-Verworn theory. The elimination is due to the total absence of any appreciable effect of the stimulating agent on the muscles of the legs. When an insect is oriented in light, the light has no immediate observable effect on the muscles.

Photic orientation in these organisms is therefore the result of series of co-ordinated reflexes in the legs of both sides, each specifically related to the location of the stimulus in either eye, and inhibition or modification of the effect of the illumination in one eye by simultaneous illumination in the other. After the insect is oriented in a field of light (no matter how complex) orienting stimulation ceases. It is not held on this course by continuous action of the light. It tends to continue in the direction assumed, owing to internal factors. But if for any reason it deviates from the course, it is immediately stimulated and turns back as described above. According to these conclusions the location of the stimuli in the eyes is quite as important in the process of orientation as the relative amount of light received by them (Mast, 1923, 1924). These views concerning the factors involved in the process of orientation are in full accord with all the phenomena presented above. They account for (*a*) orientation in insects with one eye covered; (*b*) orientation in insects with legs on one side removed; (*c*) turning upward or downward on the wing, while the two eyes are continuously equally illuminated; (*d*) turning toward the side on which the legs are more extended; (*e*) orientation in a field of light consisting of two or more beams of unequal intensity, in which the two eyes do not receive equal quantities of light after the insect is oriented; (*f*) shock-reactions in one-eyed insects; (*g*) decrease in deflexion in circus movement, with increase in light intensity; and (*h*) orientation in insects which tend to maintain an axial direction such that the images in the eyes remain constant in position. And if it is assumed that, in the fire-fly, momentary illumination of any region in the retina induces a series of reactions which, no matter where in the eye the stimulus is located, result in turning until the insect faces the direction from which the light came in place of turning through only a small angle, they also account for orientation of the male fire-fly in response to the flash of the female and for the orientation of the abdomen of the female in response to the flash of the male.

Clark (1933) maintains that the views presented are in accord with all the facts in hand concerning photic orientation in insects except three: (*a*) increase in stimulating efficiency with increase in the size of the source of light; (*b*) decrease in the angle of deflexion in one-eyed insects, with increase in time in light; and (*c*) decrease in this angle with increase in the intensity of light in low illumination.

To account for these he adds the following assumptions: (*a*) The "all-or-none" principle obtains in the stimulation of the ommatidia. (*b*) Variation in the threshold of the ommatidia in any given region is in accord with the normal distribution curve.

(c) The threshold of the ommatidia varies directly with light adaptation. (d) The magnitude of the response varies directly with the number of ommatidia stimulated.

Photic orientation in insects is as a whole adaptive. It is rarely injurious except under unnatural conditions, as, for example, in light produced by a candle in a dark room. It is instinctive in nature since it is largely independent of experience in the individual in which it occurs, and its origin and evolution are doubtless the same as those of other instinctive reactions.

(2) *Ants*

Orientation in the organisms considered in the preceding pages usually results in movement directly toward regions which are favourable in reference to environment. In the vertebrate animals, notably man, this frequently occurs without orientation, i.e. these animals can go toward any given point in space, with any part of the surface of the body ahead, and also while continuously changing the part which is ahead. The following account shows that this ability is not confined to the vertebrate animals.

Several years ago (1932 *a*) I made numerous observations on the behaviour of several different species of ants which were taking to their nests, over relatively rough terrain, particles of various kinds (bread-crumbs, pieces of dead insects and the like). It was found that if the particles were small the ants picked them up and carried them walking forward; but that if they were large, they dragged or rolled them, walking backward or sidewise; and that in going over obstacles, they often climbed nearly vertically upward and downward and frequently fell from precipices and rolled over and over; but that no matter how they progressed, they continuously proceeded in a given direction. Thus I have repeatedly seen a given individual in going toward a hole in a nest proceed forward for a moment, then backward, then sidewise, then roll over and over, then proceed forward or backward or sidewise again, etc., without any marked change in the direction of locomotion. It is therefore obvious that ants can go directly toward a fixed point in space, regardless of their axial orientation in reference to this point or the rate or extent of change in this orientation. This demonstrates conclusively that their direction of locomotion is not controlled by anything in the nature of equal or unequal stimulation or action of organs on opposite sides, correlated with the action of external agents; and it shows that they are, in some unknown way, able to compensate for the direction and rate of turning by changing the direction and the rate of movement of their locomotor appendages, just as does a human being who, with his eyes closed, goes continuously toward a given point in space while he is turning around and consequently proceeds successively forward, sidewise and backward. In man memory and reason are involved in this accomplishment. Does this indicate that they are also involved in it in ants?

VIII. DISCUSSION

The results presented above show that several factors are involved in the process of orientation in each organism studied and that these factors differ in different organisms and in the same organism under different conditions. For example they

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CONTRIBUTIONS OF CHEMICAL ~~PHYSIOLOGY~~ TO THE PROBLEM OF REVERSIBILITY IN EVOLUTION

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I. INTRODUCTION

THE conception of reversible change is one of the most fundamental in science. It is familiar in thermodynamics and astronomy, no less so in chemistry. In biology its position varies according to the subject under discussion. Biochemists are accustomed to view enzyme action, like all other chemical reactions, as reversible, according to the conditions within which the reaction takes place. The cyclical systems which have only recently been revealed as playing so great a part in metabolism involve hydrogen transporters such as cytochrome, lactoflavin and cozymase, phosphorus transporters such as adenylic acid, etc., and the only reason why such substances can act as transporters is that they can be reversibly oxidized and reduced, phosphorylated and dephosphorylated. That reversibility occurs at the level of the relatively small molecules of metabolism cannot be doubted; that it occurs at the grosser levels of the protein micelles can also hardly be doubted, seeing that these are continually broken down and built up again, and that even in their stable condition they are often able, if in fibrillar form, to exhibit contraction and extension truly molecular and beautifully reversible.

At the higher, or grosser, morphological levels, we have both ontogenesis and phylogenesis to consider. With regard to the former, the nature of the dedifferentiation occurring when vertebrate tissues are cultivated in explantation conditions may still be a matter of some obscurity, but among invertebrates degrowth and dedifferentiation are well known. In nemertines and ascidians we have classical cases of these phenomena, as well as in planarian worms.

On the phylogenetic side the position is much more obscure and the difficulties infinitely greater, for in the absence of the possibilities of experimentation, all that can be done is to measure the relics of the past and to ascertain as much as possible about the structure and function of those organisms existing to-day. The rest must always be a theoretical superstructure, and all it can hope to achieve is what has been called a "post-diction" (Woodger) advancing propositions formally unverifiable. Nevertheless this superstructure may well be regarded as among the most interesting regions of biology. Hitherto it has been built too exclusively upon morphological as opposed to chemical and physiological considerations. The conclusions of chemical physiology may be very relevant indeed to the problem of reversibility in evolution. Long ago, Lucas (1908) emphasized that physiology must contribute equally with morphology to a balanced evolution theory, and the subsequent lines of work by Barcroft (1924, 1925), Redfield (1933, 1934)¹ and their collaborators on the invertebrate blood pigments, or of Delaunay (1931)¹ and Przyłęcki (1925, 1926) on the nitrogen excretion of invertebrates, show how progress towards this aim may be made.

The general view of palaeontologists is that the phylogenetic process has marked features of irreversibility. The pioneer work of Dollo (1893, 1909) led to the formulation of the following rule (sometimes called a Law). An organ which is reduced in the course of phylogenetic development never again reaches its original importance, and an organ which has altogether disappeared never again appears. Further, if in connexion with adaptation to a new environment an organ is lost which was very valuable in the previous environment, and if a secondary return to the previous environment occurs, this organ will not reappear; in its place some other organ will form a substitute. It will be seen that this generalization (made by Dollo on the basis of a wide and deep analysis of the remains of both vertebrates and invertebrates) implies that evolution is reversible in the sense that structures which have been gained can be lost, but that it is irreversible in the sense that, once lost, these structures can never be regained.

The restriction of our present knowledge of comparative biochemistry is such that we can hardly hope to derive from it as yet any crucial evidence on such a generalization as this. Nevertheless certain data force us to consider its validity in a realm where enzyme systems and physiological processes, rather than the shapes of bones or the presence or absence of certain morphological structures, are studied. The purpose of the present review is to bring together some of these data, and we shall take up in due order (1) the enzyme equipment of bacteria and Protozoa, (2) the physiological uraemia of elasmobranch fishes, (3) the nitrogen catabolism of vertebrates and invertebrates, particularly the uricotelic habit in molluscs, (4) the water metabolism of the cleidoic egg, and (5) the chemical physiology of the teleostean fish kidney.

Before entering upon the detailed discussion of these cases, however, something must be said about the classical morphological examples of Dollo's Law. No purpose would be served by a minute description of these, especially by one who would

¹ *Biological Reviews*.

be the last to claim any competence in palaeontology, but there may be other biologists who would welcome a reminder of them. We shall follow the writings of Abel (1912), of Haecker (1922), who has devoted a special monograph in Schaxel's *Abhandlungen* to the subject, and of Petronievics (1918), whose interesting but often forgotten article contains a very useful bibliography of Dollo's papers. Petronievics distinguishes between "progressive" evolution, in which a continual gain in organs and functions is going on, and "regressive" evolution, in which a continual loss in them occurs, as in the assumption of parasitic habit. Most cases of evolution are neither the one nor the other, but mixed, in which case if the net results are gains we may speak of "ascending" evolution, if losses of "descending" evolution. We may usefully employ these terms in the present article. The foot of the horse, originating from a pentadactyl appendage, would be an example of mixed ascending evolution, and the transition in the lung fishes from the skull of *Dipterus* to that of *Ceratodus* (Dollo, 1895) would be one of mixed descending evolution.

The pseudo-dentition of *Odontopteryx*, an eocene bird, is one of the best-known cases of Dollo's Law. Instead of the lost teeth, which apparently it cannot regain, its beak and lower jaw are serrated in saw-like form. Again, the changes in the pelvis of the dinosaurs are examples, for in secondary adaptation to quadrupedal life, the ischium, postpubis, etc., conserve the very apparent traces of their modifications to earlier bipedal existence (Dollo, 1905). Then the Octopoda, in adapting themselves to a benthic life on the sea bottom, lost the tentacular arms of their immediate ancestors, the heteropodous Decapoda. They thus became isopodous, like their remoter ancestors, the belemnoteuthids, but with a smaller number of arms (Dollo, 1912). The structure of the kangaroo foot shows the same irreversibility. The foot of the Macropodidae betrays the fact that their ancestors were tree-living forms by the dominance of the fourth toe, the syndactyly of the second and third, and the reduction of the fifth. Later, the big toe completely disappeared. But in *Dendrolagus*, a form which has made a secondary return to tree-living life, the opposable big toe of its remote ancestors has failed to reappear; on the contrary, the ends of the digits have elongated and turned into long curved claws (Dollo, 1899). Lastly, the development of secondary armour in *Dermochelys* is a classical case of irreversibility (Dollo, 1901). The distant ancestors of this turtle were marine, as is shown by the highly reduced primary carapace and plastron (the former nothing but a small nuchal plate). In adapting themselves to secondary littoral life, the ancestors developed carapace and plastron again, but from a quite new, dermal, superficial origin. In a tertiary return to the sea, these new coverings are themselves again reduced. For extended discussion of these and other instances, the papers of Petronievics (1918) and Nopcsa (1923) should be consulted.

Nopcsa raises some doubts about the universal validity of the generalization. He considers five cases of evolutionary change in reptiles: (a) the elongation of the anterior limbs (in specialized dinosaurs), (b) the development of the post-orbital bar, (c) the development of the ventral elements of the pelvis, (d) the changes in the carpus and tarsus, (e) the development of the supra-orbital region. He concludes that the first is a case of reversible evolution, since it shows how an ossification,

which was interrupted during a certain period of adaptation, sets in again. The second and third cases show the persistence of a primitive stage of development in later more specialized forms and the subsequent development of another stage of evolution through which the ancestral forms had passed long ago. The last case shows three phases, first, one in which a character is not yet fixed so that a reversal is possible; secondly, an undetermined phase; and thirdly, a set phase in which reversal is impossible. In this last phase a particular function can only be attained by the development of a new organ. Whether Nopcsa's cases really transgress the limits of Dollo's Law, and whether his conception of instability followed by stability has any relationship with the parallel progress of determination in embryonic development, are questions which only the palaeontologists can answer. Nopcsa suggests that, besides the assumption of a function by a new organ or part after the old one has irreversibly disappeared, organisms may be able to call upon qualities possessed by embryonic stages, preserving them into adult life. This is that "paedomorphosis" about which de Beer (1930) has so well written.

Other instances of irreversibility may be taken from regions of the animal kingdom far distant from one another. In the colonial hydroids there is a tendency for the originally free-swimming *Medusa* to become more and more reduced to the condition of a sac which never becomes detached and free. If in some cases, such as *Dicoryne*, the sac becomes detached, regaining its mobility, it cannot be mistaken for a *Medusa*, because the muscles have disappeared, and for locomotion the sac has to have recourse to a different means altogether, cilia. As de Beer says, "while structural reversibility appears never to have taken place in evolution, functional return to a previous condition, using other instruments, is not uncommon".

Brilliant examples of this are to be found among the insects. The larval Diptera are all alike in that they never possess legs, and yet employ every conceivable method of locomotion, using modified mandibles as legs, moving in vermiform manner, or jumping by violent muscle contractions. The fact is made even more remarkable by the fact that, in the form of imaginal discs, leg rudiments are present all the time. Keilin (1915 *a*) explains these phenomena by suggesting that all modern diptera are derived from ancestors whose larvae were either parasitic, xylophagous, or occupying some position where locomotion was unnecessary. But further the cyclorhaphous dipterous larvae (such as that of the flesh-fly) as opposed to the orthorhaphous types (such as that of the mosquito) have, in effect, lost their heads as well as their legs. It is therefore striking, as Keilin (1915 *b*) points out, that a far larger proportion of cyclorhaphous larvae are parasitic than orthorhaphous ones, among which, indeed, parasitism is rare. Consider also the frequent occurrence of larviparity and pupiparity among the cyclorhaphids, analogous in many ways to parasitism, and furthermore, the enormous extent of the saprophage habit among them, bordering indeed on parasitism in those species which produce the myiasis diseases.

Gnat larvae also provide, as Keilin (1924) has shown, remarkable evidence for evolutionary irreversibility. When dipterous larvae pass from terrestrial to aquatic existence in the course of evolutionary change, they generally concentrate their respiratory apertures at two points at each anterior or posterior end of the body,

leaving thus the peripneustic condition and becoming amphipneustic. These two points develop as siphons from which the larva may hang from the surface of the water. It is therefore of much interest to find that many syrphid larvae, which have made a secondary return to terrestrial conditions, still possess their siphons and amphipneustic spiracles, although the adaptational value of these has long disappeared. But the peripneustic condition may also give place to the apneustic condition when an aquatic larval life is taken up; here the larva relies on dissolved gases in the water, and possesses no spiracles at all. Nevertheless, Keilin found an instance of failure to recede even from this extreme type of specialization when a secondary return to terrestrial life had been made. *Forcipomyia*, a ceratopogonid gnat belonging to the group of chironomids, is terrestrial in the larval stage, but yet quite apneustic. In accordance with Dollo's Law it has had to have recourse to further modifications and has developed hairs on the end of each of which is some mechanism of deliquescence, so that drops of water continually cover the surface of the larva, permitting the absorption of dissolved gases.

Reversion from parasitism, with consequent failure to regain lost organs and functions, has been invoked by Keilin (1926) as an explanation for the great difficulty which systematists find in determining the affinities of the nematodes with other invertebrate groups. The view that a kinship with arthropods exists, expressed by Baylis (1924), is by no means convincing, and Keilin suggested that the free-living nematodes of to-day (far less in number than the parasitic ones) are really descendants of groups all of which were parasitic. The entire phylum has thus lost all the structures which could allow us to discover its affinities with other phyla. If this is so, a biochemical and physiological problem of no small interest is presented to us. The parasitic nematodes are characterized by very peculiar metabolic properties, among which may be mentioned the excretion of large amounts of valeric acid as end-product, and the uncommon use of waxes and sterols (see the recent papers of Krüger, 1935, 1936). A biochemical examination of the free-living nematodes is urgently called for.

If, then, for the present purpose, we may take it that *evolution is reversible in that structures or functions once gained may be lost, but irreversible in that structures or functions once lost can never be regained*, we shall see that the biochemical examples to be given are in general agreement with this. Examples of the first proposition are the enzyme equipment of unicellular organisms, the uraemia of elasmobranchs, and the uricotelism of molluscs. Examples of the second proposition are the water metabolism of the cleidoic egg of the sauropsida, and the glomerulus of the teleostean fishes.

II. THE ENZYME EQUIPMENT OF UNICELLULAR ORGANISMS

A great deal of biochemical work upon the bacteria has led to the view that evolutionary adaptation has here been accompanied by widespread loss of functions primitively possessed. In a recent review Fildes (1934) points out that the autotrophic bacteria must be regarded as the most primitive. They are bacteria which

can obtain their energy and building material from purely inorganic sources, requiring no co-operation of organic matter or other life. The autotrophic bacteria (Winogradsky, 1931; Stephenson, 1930) can oxidize substrates such as hydrogen, ammonia, carbon monoxide, hydrogen sulphide, sulphur, iron, etc., and with these simplest sources of energy can construct protein and other necessary molecules from carbon dioxide and ammonia. "These inorganic materials", writes Fildes, "would be present in the muddy swamps (prior to the appearance of any other forms of life) where the autotrophic bacteria could have been the first forms of life to develop. They could have existed before any vegetable matter requiring a photosynthetic mechanism because they could have existed before the sun's rays penetrated the overhanging clouds—before there was light."

The primitiveness of the autotrophic bacteria need not be further emphasized. At the other end of the scale from them we find bacteria such as the organisms pathogenic to the higher animals and man. Thus the typhoid bacillus, as Fildes *et al.* (1933) have shown, normally requires the presence of tryptophane in the medium. It is not capable of synthesizing this amino acid from ammonia. It cannot use ammonia as its sole source of nitrogen. Such a case as this—and there are many others—may therefore be interpreted as meaning a loss of function consequent upon the adoption of the parasitic habit.

It is remarkable, however, that this missing enzyme can be fairly readily reformed in the bacillus, contrary to what would be expected if bacteria always behaved according to Dollo's Law. Knight (1936) describes work which shows that the typhoid bacillus can be trained to synthesize tryptophane from ammonia if a strain is gradually acclimatized to its absence from the medium. As this acclimatization goes on, virulence diminishes. This is a case analogous to those of "adaptive enzymes" which have been studied by Stephenson (1932, 1933, 1934, 1935) (cf. Stephenson & Yudkin, 1936; Passmore & Yudkin, 1937), and reviewed by Karström (1930) and Yudkin (1938).¹ When a given bacterium is grown in the absence of formate it will not possess enzymes capable of oxidizing formate, but the same organism grown in the presence of formate *will* manifest the existence of such enzymes. The mere presence of an unusual substrate will bring about the appearance of enzymes capable of dealing with it. As Stephenson & Yudkin have shown, this process cannot be explained by a kind of natural selection of individual bacterial cells, but must involve specific reactions on the part of most of the population.

Absolute irreversibility in the bacteria, then, is not fully present. Nevertheless, no case has so far been recorded of a reversion naturally or experimentally produced, from a highly specialized, perhaps pathogenic or saprophytic bacillus, to the primitive autotrophic type, capable of deriving energy from the oxidation of sulphur or iron. The nutritional requirements of the pathogenic bacteria, moreover, are often surprisingly specific. Barron & Miller (1932) have shown that the *Gonococcus*, for example, has a poor and ineffective range of enzymes. The tubercle bacillus can use few sources of energy and nitrogen. Cozymase is known to be indispensable for the growth of the influenza bacilli, which cannot synthesize it themselves (Lwoff, 1936).

¹ *Biological Reviews.*

But we need more comparative surveys of the synthetic powers of related pathogenic and non-pathogenic bacteria.

The Protozoa offer at least as striking examples of loss of chemical functions as the bacteria, and this regressive specialization has been made the subject of an interesting monograph by Lwoff (1932). He follows the course of an evolution from the chlorophytes to the Protozoa in which many stages of physiological degradation can be distinguished. Physiological degradation he defines as the passage from a higher to a lower synthetic capacity, and this change may be quantitative, i.e. a diminution of function, as well as qualitative, i.e. the disappearance of the function. Nitrate, ammonia, and amino acids constitute a series of substrates, the first of which offers most difficulty as a building material for the construction of the body protein, the second less difficulty and the third least. The primitive Chlorophyta possess the property of utilizing nitrate as sole source of nitrogen, the Leucophyta require either ammonium salts or amino acids, and the needs of the Protozoa are still more special; they must have complex polypeptides and cannot use one amino acid or even an amino-acid mixture. In the *Euglena* group a number of stages can be found (Dusi, 1933). *E. stellata* is capable of making good growth on nitrate as sole source of nitrogen; *E. gracilis* can do so, but not very successfully; for *E. anaerobica*, even in the light, ammonium salts are essential; while for *E. deses*, even in the light, nothing less than amino acids will do. Finally, when *E. gracilis* is cultured to a stable race in darkness, it loses all power to use nitrogenous substances of lower complexity than peptones. This position approximates to the requirements of the protozoa *sensu stricto*, such as *Glaucoma* (probably the only holotrichous ciliate which has ever been studied biochemically in medium free from bacteria), which can make use of no source of nitrogen less complex than peptones. The last stage of this biochemical regression is occupied by those parasitic bacteria and flagellate Protozoa which need, in addition to peptones or polypeptides, special activating substances only found in the blood of their hosts.

The classical examples of this last stage are undoubtedly the trypanosomes. In remarkable work, M. Lwoff (1933) and A. Lwoff (1934) have conclusively demonstrated that the missing factor without which the parasitic trypanosomes *Strigomonas* and *Leptomonas* cannot respire and multiply is the tetra-pyrrol ring system of haemoglobin and cytochrome. Growth of *Strigomonas* in peptone solution is proportional to the amount of added blood, 1.10^{-12} g. blood allowing six cell divisions. Respiration, much reduced in the absence of blood, is restored by 1.10^{-5} g. blood for each mg. dry weight of flagellates. Of all the substances tested as substitutes for blood, protohaemin and protoporphyrin alone would act, and it was concluded that each flagellate lacks, in the absence of blood, 520,000 molecules of protoporphyrin from which to synthesize the cytochrome and perhaps the *Atmungsferment* necessary for its respiration and multiplication.

The information described above is summarized in the accompanying table, where the loss of synthetic capacities is well seen. This loss may manifest itself not only in the anabolism of nitrogen, but also in other metabolic departments, such as that of sulphur (cf. the need for —SH or —SS on the part of the mould *Sapro-*

legnia). Krijgsman (1936), who reviews the whole field of trypanosome metabolism, shows how restricted and simplified its activities are. In *Trypanosoma evansi* lipases and amylases are quite absent, and of the proteases only kathepsin is found. There is no catalase and no urease. Glycolysis probably takes a non-phosphorylating course (Reiner *et al.* 1936).

Table I. *Regressive evolution by loss of synthetic capacities among the Protista*

Procaryota	Eucaryota			Food				
	Chlorophyta	Leucophyta	Protozoa	Nitrate	NH ₃ salts	Amino acids	Peptones	Peptones + special activators
Bacteria, <i>Aspergillus</i>	<i>E. stellata</i> (in light) <i>E. gracilis</i> (in light)	Polytoma		+	+	+	+	+
Bacteria, <i>Saccharomyces</i>	<i>E. anaboena</i> (in light) <i>Chlamydomonas</i> and <i>Haemato-</i> <i>coccus</i> (in dark- ness) <i>E. deses</i> (in light) <i>E. gracilis</i> (in darkness) <i>E. pisciformis</i> (in light)			±	+	+	+	+
				o	+	+	+	+
				o	o	+	+	+
				o	o	o	+	+
Parasitic bacteria			Parasitic flagellates, <i>Leptomonas</i>	o	o	o	o	+

Note. (1) The classification of Chatton (1926), used in the above table, divides the Protista into Procaryota (without nucleus or mitochondria) and Eucaryota (with these structures); further, the Eucaryota into the Chlorophyta (possessing plastid and chlorophyll), the Leucophyta (possessing plastid only), and the Protozoa *sensu stricto* (possessing neither).

(2) *Glaucoma* is the only protozoan which has so far been maintained solely on dissolved substances, but experiments with *Acanthamoeba* indicate that the nutritional requirements of those which need solid particles are similar to those of *Glaucoma*.

“L'Évolution physiologique des organismes”, writes Lwoff, “se traduit par une perte successive des fonctions. Ces pertes de fonctions sont irréversibles.” In the disappearance of the plastid, and the subsequent disappearance of the power of synthesizing the tetra-pyrrol ring system, we witness the beginnings of all the three sciences, botany, zoology, and parasitology. It appears that some instances are known where this irreversible loss of functions has been induced experimentally. In 1883 Chamberland & Roux found that *B. anthracis*, cultivated in bichromate broth, lost permanently the power of spore formation, and it has since been shown that this holds true for at least 5 years. Similarly, in 1892 Gessard obtained a new race of *B. pyocyaneus*, unable to produce its usual bluish green pigment, by cultivating it for a year in liquid egg-white medium. This race has been cultivated for 39 years in the Pasteur Institute at Paris, and retains its incapacity to this day.

In general, then, we see, in the regressive evolution of the unicellular organisms, a reversibility in so far as they lose their primitive synthetic powers, and an irreversibility in that they never can regain them.

III. THE PHYSIOLOGICAL URAEMIA OF ELASMOBRANCH FISHES

The presence of large amounts of urea in the tissues, blood, and eggs of elasmobranchs is now a very old observation. In 1858 Stadeler & Frerichs isolated "kolossale Quantitäten von Harnstoff" from the organs of plagiostomes, obtaining a solid mass of urea nitrate when they added nitric acid to their final concentrates. These workers also showed that no such large accumulations were to be found in teleosts or cyclostomes. Since that time there has been abundant confirmation of these results by many investigators, so that Smith *et al.* in 1929 could definitely say that "high blood-urea is a phyletic character of the orders Selachii and Batoidei".

The significance of this urea was only slowly understood. The work of Rodier (1899), Duval (1925) and others showed that, whereas the ash alone of elasmobranch blood corresponded only to an osmotic pressure of $\Delta - 1.06^\circ$, the whole blood was just hypertonic to sea water (e.g. *Torpedo* $\Delta - 2.26^\circ$; *Trygon* $\Delta - 2.44^\circ$; sea water $\Delta - 2.1^\circ$). The urea has therefore come to be generally regarded as a device for raising the osmotic pressure of the internal medium above that of the external medium, and so ensuring the existence in the sea of that favourable osmotic gradient which all inhabitants of fresh water enjoy.

The contrast between this method and that adopted by the marine teleosts is very great. In a classical paper Smith *et al.* (1930*a*) showed that marine teleosts continually swallow relatively large quantities of sea water which subsequently undergoes absorption through the intestinal walls. Most of the ingested water, sodium, potassium and chloride, are absorbed, leaving a residue rich in magnesium and sulphate and apparently isotonic with the blood, to be excreted at the anus. The osmotically dilute nature of the intestinal residue and the urine shows that osmotic work is being done at some point other than the kidneys or the gastro-intestinal tract, and, in fact, it is found that an important extrarenal excretion of sodium, potassium and chloride is going on by way of the gills. This gill excretion is hypertonic to the ingested sea water, while the urine, though always small in amount in marine teleosts, is hypotonic. From this complicated mechanism of swallowing and osmotic work at the gill membrane, the elasmobranch, with its high internal osmotic pressure, is entirely free (Smith, 1931*a*, 1936).¹

These facts bear upon the problem of evolutionary reversibility because of the interesting circumstance that some *fresh-water* elasmobranchs exist at the present day. Smith & Smith (1931), who made a physiological study of those in the Siamese rivers, list twenty-two species of them. Although the elasmobranchs are predominantly marine to-day, there are reasons for supposing that their marine habitat is secondary and that those of the Silurian-Devonian periods were largely and primitively inhabitants of the continental fresh waters. "There are no reasons for

¹ *Biological Reviews.*

supposing", wrote Smith & Smith, "that any elasmobranch, under proper oecological conditions, might not perform a migration back to the ancestral home of the race."

But if this occurred, what would happen to the accumulation of urea in the blood, now no longer of adaptive value? If evolution were irreversible, the extremely high urea retention would persist, since the actual height of the osmotic gradient will matter little provided it is favourable, but if it were reversible the urea retention might altogether disappear. Fig. 1 shows that the reversibility is in effect a quantitative one, for the urea-content of the fresh-water elasmobranch is still high in comparison with other animals, although much reduced in comparison with the related marine species (av. 1000 mg. per cent marine elasmobranchs; 300 mg. per cent fresh-water elasmobranchs, 20 mg. per cent other organisms). "When viewed in relation to the blood of teleosts, the only significant difference in composition of the blood of these fresh-water elasmobranchs is the persistence, though at a much lower level, of the uraemia which characterises the marine forms. It should be noted that the urea is reduced to a much greater extent than the total osmotic pressure or the chloride. This fact suggests that the uraemia bears a special relation to a marine habitat, but that when the elasmobranch invades fresh water, the uraemia is not entirely obliterated" (Smith & Smith).

The Elasmobranchii almost alone among animals discovered how to withstand the accumulation of great quantities of urea within their tissues without toxic effects. But they were not quite alone in this, for a similar adaptation is found amongst the ancient group of lung fishes, the Dipnoi. Here again, physiological work has been done by Smith and his collaborators (1930*b*), who found that, during aestivation, when the lung-fish such as *Lepidosiren* or *Protopterus* is asleep in its mud burrow, it continues to derive most of its energy from the combustion of protein. This leads, not to the formation of uric acid, but to urea, progressively saturating its tissues until a concentration of up to 2 g. per cent is reached (quite similar to the elasmobranchs), after which, on waking and leaving the burrow, a spectacular excretion occurs. The significance of the physiology of these fishes from the point of view of early efforts at terrestrial life, and the evolution of the Amphibia, needs no emphasis, but it is remarkable that harmless uraemia is to be found only in these two groups of fishes (Smith, 1931*b*). It may be supposed that what was an adaptation to life in concentrated external medium for the Elasmobranchii became an adaptation to aestivation for the Dipnoi.

Adopting for the present the belief of Chamberlain (1900) and Barrell (1916) that the palaeozoic chordates evolved in continental fresh waters, and that they subsequently spread into the sea after the Silurian-Devonian periods, we have seen that two methods were used: (*a*) the sea-water ingestion and hypertonic branchial excretion of the teleosts, (*b*) the uraemia of the elasmobranchs. Another alternative would have been to raise the internal osmotic pressure not by a small nitrogenous molecule, but by ash, above the exterior. This course appears to have been taken by the Myxinoidea, if we may judge from the work of Bond *et al.* (1931), Smith (1932) and Galloway (1933) on the hag-fishes *Myxine* and *Polistotrema*. Borei (1935),

however, considers that this condition is mixed with some activity of the typical teleostean mechanism, since he finds the internal osmotic pressure of *Myxine* to be slightly below that of the sea water. In any case, this hypertonicity or isotonicity does not appear in the lamprey, *Petromyzon*, which in sea water resembles the marine teleosts. Palaeontologists believe that the Petromyzontia evolved either from the Anaspida or from the common ancestors of these and the Osteostraci, whereas the Myxinoidea are descendants either from Palaeospondylus or from the primitive Heterostraci. Smith comments that these two groups may lead back to a parting of the ways in the evolution of body-fluid regulation.

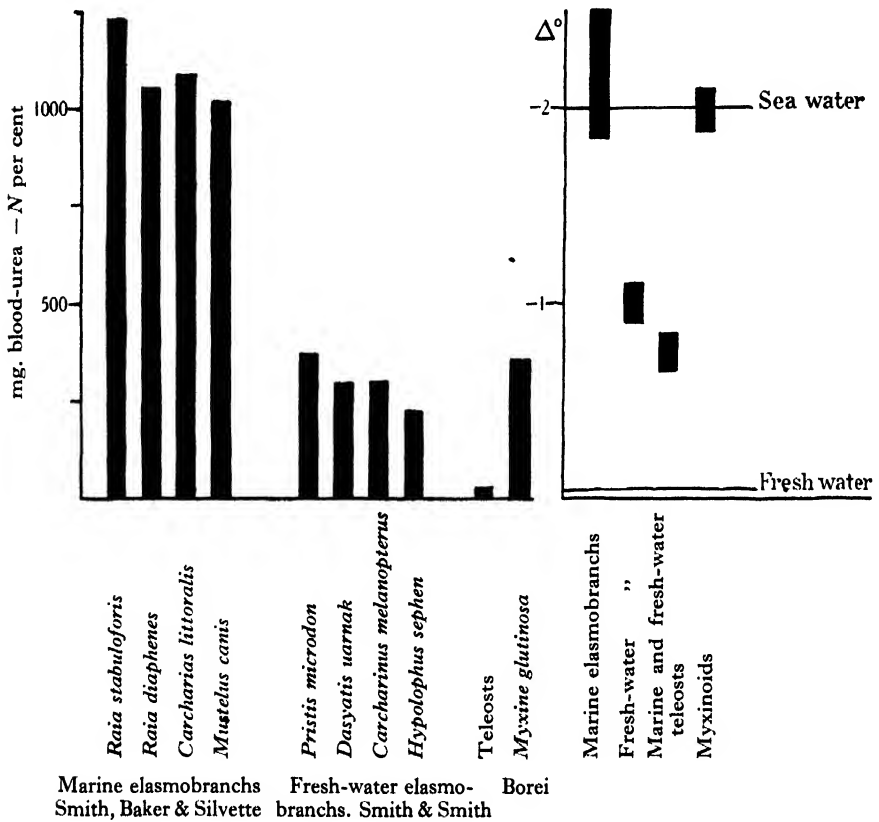


Fig. 1.

The argument of this section is, then, that in the decrease of blood urea in fresh-water elasmobranchs we see an instance of regressive (and hence reversed) evolution. But a further consequence follows with respect to the Myxinoidea. Borei (1935) made the remarkable discovery that the blood of *Myxine glutinosa* (the marine cyclostome) contains 360 mg. per cent urea, that is to say, an amount strictly comparable with that found in the blood of the regressed fresh-water elasmobranchs (see Fig. 1). Unlike them, however, its total internal osmotic pressure is made up to the value of the external sea water by inorganic salts. Now if Dollo's Law held

good, we should expect that the tertiary return of a fresh-water elasmobranch to sea water might well occur, but if it did, the urea-retention mechanism would not be used again. Would it be permissible to see in *Myxine* a case of just this sort; first the development of urea retention for marine life, then the virtual loss of it in fresh-water existence, and finally the replacement of it by another mechanism (salt retention) when the animal again became marine? On this interpretation *Petromyzon* has never been marine, but *Myxine* has been so twice. Efforts directed to studying the urea content of the blood of all available Cyclostomata would probably be well worth while. There is no reason why certain divisions of this primitive group should not have made use of urea retention just as did the less primitive descendants of the ostracoderms (Selachii, Batoidei, Chimaeroidei), and even also (if in transient fashion) the most primitive bony fishes (Dipnoi). The interpretation proposed here, moreover, is in agreement with the fact that the glomerular apparatus of the cyclostome kidney (Conel, 1917, for *Bdellostoma*) resembles that of the elasmobranchs; it never disappeared because urea retention made oliguria unnecessary. But the significance of this fact will appear in a later section.

IV. THE URICOTELISM OF TERRESTRIAL MOLLUSCS

There are in the animal kingdom three main ways of excreting waste nitrogen from protein catabolism—ammonia, urea, and uric acid. In addition to these substances, many invertebrates excrete amino acids unchanged, as if the metabolic machinery of the body which separates the amino acids of the food into quotas for storage and quotas for combustion were inefficient (Delaunay, 1931). In certain special groups also there may be special methods or adaptations, such as the excretion of guanine by arachnids (Vajropala, 1935) and trimethylamine-oxide by certain teleosts, but these may legitimately be omitted from a general survey (Grollmann, 1929; Grafflin & Gould, 1936).

That the elaborate process of the formation of the purine ring from the catabolic nitrogen had something to do with terrestrial life was early surmised, but the mammalia, which excrete the whole of their waste nitrogen in the form of urea, were an insuperable obstacle to so simple a generalization. In a series of communications (Needham, 1929; 1931, § 9) it was suggested that the form of nitrogen excreted by an animal depended primarily on the conditions under which its embryo had to live. It may be said that all the data which have come to light since that time have supported the suggestions then made. They may be summarized as follows. Between uric acid excretion¹ and terrestrial oviparity there is a strict correlation, ammonia and urea being associated with aquatic pre-natal life (including development within the mammalian uterus) and uric acid with terrestrial pre-natal life. An inspection of the tables given in the above papers showing the percentage nitrogen partition in the excreta of representatives of every phylum shows the strictness of the corre-

¹ It must be understood that we are speaking here only of uricotelic excretion, i.e. the excretion of uric acid as the main end-product of nitrogen metabolism. Uric acid as the end-product of nuclein metabolism is a quite separate, and minor, phenomenon.

lation. Birds, saurian reptiles, and insects alike possess the uricotelic habit; mammals, chelonian reptiles, Amphibia and fishes excrete their waste nitrogen mainly as ammonia or urea.

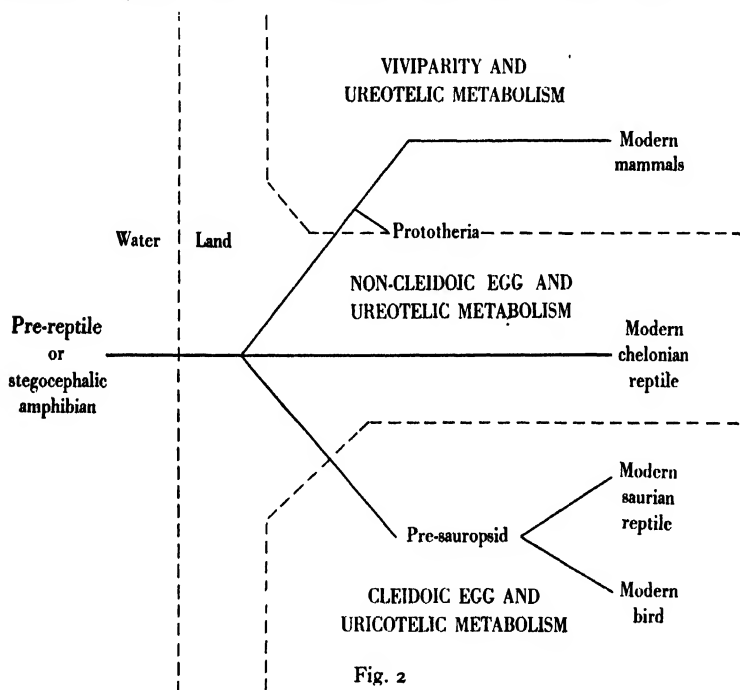
Connected with this was the generalization that some animals have eggs much more completely closed off from their environment than others, and the word "cleidoic" (closed box) has come into use to designate the former. A typical non-cleidoic egg would be that of a cephalopod, which is not supplied by the maternal organism with sufficient ash or sufficient water to make one complete embryo, but has to absorb many of its constituents from the sea (Ranzi, 1930). The mammalian egg must also be regarded as non-cleidoic, for even its organic constituents are not stored in it at the beginning of development, but have to be provided later on from the maternal organism. A typical cleidoic egg, on the other hand, would be that of a bird, where all that the embryo requires, except the oxygen, is provided within the closed system. Between these extremes many intermediate grades are found.¹

Far-reaching metabolic changes are associated with the evolution of the cleidoic egg, for closure of the egg-system is accompanied by difficulties in the disposal of incombustible nitrogenous waste products. Thus it has been shown that the eggs of birds, reptiles, and insects combust markedly less protein during their embryonic life in per cent of the protein initially present than do the eggs of aquatic animals such as fishes or amphibia. The figures are of the order of 3 per cent in the former as against 25 per cent in the latter. Similarly, of the material burned as source of energy during development 6 per cent is protein in the case of the chick, but 71 per cent in the case of the frog. On the other hand, the catabolism of fat is correspondingly increased in cleidoic eggs, so that, whereas the aquatic eggs of amphibia and fishes make it some 20 per cent of their total combustions, the cleidoic ones of birds and insects make it always over 80 per cent.

The cleidoic condition may also be connected with other peculiarities, perhaps the most striking of which is the rapidity of avian incubation time as compared with mammalian gestation time, i.e. the fact that it takes considerably longer to make a mammal of given birth weight than a bird of equivalent hatching weight (Needham, 1930). For a weight of 1 g. the mammalian time is 20 per cent longer; for a weight of 10 or 100 g. it is twice as long, and for a weight of 1 kg. it is three times as long. It is suggested that this is an adaptive acceleration of the developmental time, permitting rapid passage through the cleidoic state.

¹ Smith (1932, p. 16; 1936, p. 74) is reluctant to accept the view that uricotelism and terrestrial oviparity are related, but he consistently avoids the difficulty that no other generalization will explain the lack of uricotelism among the mammals, a plainly terrestrial class. It may be granted to him that the toxicity of urea has been somewhat overrated in the past, but the impossibility of the alternative of urea excretion need not have been the sole factor leading to uricotelism. A correlation has been suggested between urea retention and cleidoicity (Needham & Needham, 1930; Smith, 1936). The only class of animals lower than the reptiles, it has been said, which have evolved a structure resembling the cleidoic egg are precisely the elasmobranchs. This may be viewed as an adaptation enabling the embryo to retain its maternal and its own urea in preparation for its adult marine life. Or, alternatively, it may be said that the survival value afforded by the protection of cleidoic egg envelopes was only a possibility in animals for which the disposal of nitrogenous end-products during development was, for other reasons, no problem. But the whole correlation is rather unconvincing, since some invertebrates, such as the cephalopods, have elaborate egg cases and yet show neither urea retention nor uricotelism.

On these views, as has already been said, the mammalian embryo is an aquatic organism, its metabolism being adjusted to unlimited water intake and to the easy removal of waste products (by means of the maternal alimentary canal and the placenta and maternal kidney respectively). It is therefore not surprising that mammals are urea-producing organisms. And when we come to consider the phylogenetic relationships involved, we find they can be represented diagrammatically in a scheme somewhat similar to that shown in Fig. 2. The ornithine cycle (Krebs & Henseleit, 1932), by means of which urea is made from catabolic nitrogen in the mammals, reaches back, we know, into the realm of the fishes and Amphibia (Manderscheid, 1933); and it is therefore extremely probable that this was the



manner in which urea was formed by the primitive pre-reptiles or stegocephalic Amphibia from which modern mammals and modern Sauropsida are alike derived. There were then at least four possibilities open: (1) to take on a terrestrial life, but by adopting true viviparity to retain the ancient system of urea production; this led to the mammals of the present day; (2) to take on a terrestrial life and to make use of the completely cleidoic egg, thereby passing to uricotelic metabolism, as did the Sauropsida; (3) to retain a semi-aquatic mode of existence and to continue to lay non-cleidoic eggs outside the body; this led to the modern Chelonians; (4) to burn no protein at all during embryonic life and hence to avoid the problem of incombustible toxic nitrogenous residues. This course alone would have permitted the existence of the terrestrial cleidoic egg without uricotelic metabolism, and perhaps some of the extinct saurians explored the possibilities in this direction, but as far as our knowledge goes to-day, life without protein combustion is impossible, and

nothing seems to have come of such experiments. The three courses actually taken are illustrated in Fig. 2.

The position may be stated in another way by referring to Przylęcki's rule (1926), which observes that in the animal kingdom uricase (the enzyme which breaks down uric acid) and uricoligase (the enzyme complex which forms it from catabolic nitrogen) are never found together. This is an important support for the view already expressed, that uricotelic metabolism is essentially an adaptation. That it is an adaptation to terrestrial oviparity follows from other considerations. It is of particular interest that during the first week of the chick embryo's development, an active uricase is present (Przylęcki & Rogalski, 1928), but this soon disappears and the characteristic uric acid production supervenes. It is difficult to avoid seeing in this a phenomenon of recapitulatory significance, since uricase is present in all fishes and Amphibia, transforming the uric acid of their purine catabolism into oxalic acid and urea (Stransky, 1933). Some ammonia and urea is also formed during the chick embryo's development, and here also a recapitulatory significance is likely, since ammonia is the typical excretory product of many invertebrates and of teleostean fishes, while urea is, as we have seen, characteristic of Amphibia (Needham *et al.* 1935). The aquatic larvae of insects, too, according to Fox (1937), excrete ammonia instead of the uric acid formed by the adults, and so do the larvae of muscid flies, which in the early stages even possess a uricase like that of the chick embryo (Brown, 1936; Brown & Farber, 1936).

We now come to the bearing which these facts have upon the question of phylogenetic reversibility. The birds and saurian reptiles, as at present existing, do not enter the argument, for none of the Sauropsida can be said to have adopted an entirely aquatic mode of life after their earlier profound specialization for terrestrial or aero-terrestrial conditions. But there is another group of organisms some members of which have made a secondary entry into an aquatic environment after having become almost completely adapted to a terrestrial one, including the acquirement of uricotelic metabolism. This group is that of the gastropod molluscs.

Strohl (1914), in his valuable review of the excretory mechanisms of molluscs, came to the general conclusion that the gastropods are characterized by positive findings of uric acid in their nephridia, but that in the lamellibranchs this substance is always absent, and its place taken by urea. This is obviously in rough agreement with all that has so far been said, seeing that the former embody the large class of terrestrial pulmonates and that the latter are wholly aquatic. But this generalization rested only on qualitative tests scattered through the literature, unsatisfactory because a positive uric acid test might imply no more than the presence of a nuclein catabolism of the usual type and nothing about the presence of uricotelic metabolism. It remained for later investigators (Wolf, 1933; Baldwin & Needham, 1934; Grah, 1937) to demonstrate with varying degrees of success by *in vitro* experiments that the snail does actually form uric acid from added ammonia or amino acids.¹

¹ Edson *et al.* (1936) have shown that hypoxanthine is an intermediate in the uric acid synthesis of birds. Whether this synthesis follows the same chemical course in birds, insects and molluscs is as yet an unsettled question.

That the terrestrial pulmonate gastropods were really uricotelic, however, had long been probable by reason of the extraordinary accumulations of uric acid in their nephridia. Quantitative estimations give figures such as 660, 720, and 810 mg. per g. dry weight for the nephridium of *Helix pomatia*, so that no less than three-quarters of the dry weight of the organ may consist of uric acid. Starting from this point it was thought worth while to make a comparative survey of the uric acid retentions of many gastropods (Needham, 1935) in the hope that the presence of uricotelic metabolism might reveal itself by high uric acid retentions.¹ It must be emphasized that in the absence of information regarding the speed of excretion in

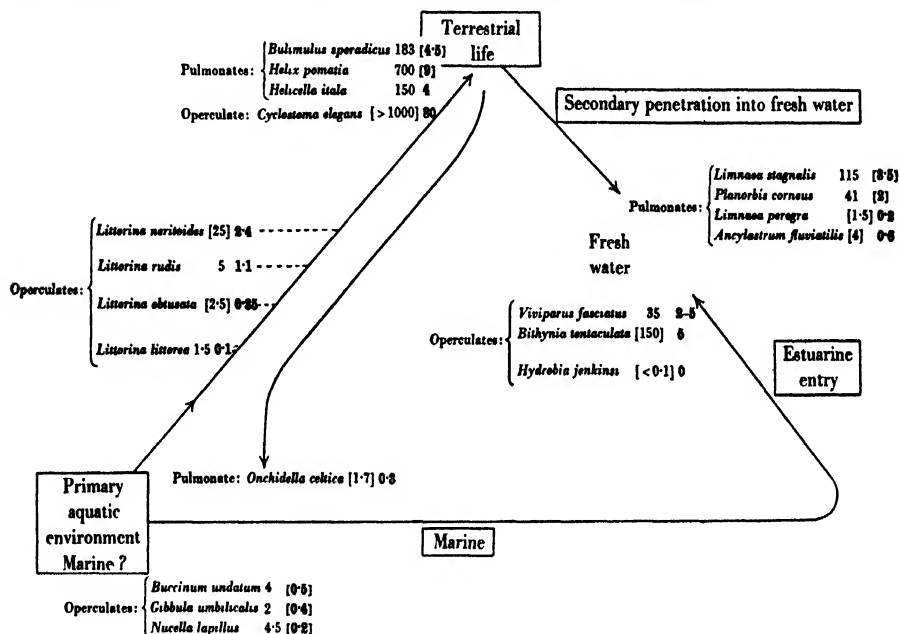


Fig. 3. Uric acid retentions in gastropod molluscs (mg./g. dry wt. nephridium in ordinary type, whole body in black type).

N.B. The figures in brackets are calculated from a graph made by plotting nephridial figures against whole-body figures on double log paper: the rest are all experimental.

each of the species studied, the results cannot be made the basis of any final scheme, but there are reasons for believing that the excretory rate of all molluscs is very slow (Cuénot, 1892, 1894, 1899; Strunk, 1935; Florkin, 1935; Picken, 1937). Moreover, the consistency of the data make their examination from the evolutionary point of view worth while, especially if we take this example as an instance of the way in which comparative biochemistry may contribute to the problems of evolution rather than as a conclusion fully worked out for one special field.

The general results are conveniently summarized in Fig. 3. Beginning with eight species of marine gastropods (operculates) represented as occupying the primitive aquatic environment at the left side of the diagram, it was found that in

¹ The subsequent work of Spitzer (1937) overlapped with this to some extent; a comparison of the two sets of figures reveals very considerable agreement.

all cases their uric acid content was very low¹ (N. 2-4½, W.B. 0.2-0.5). This was no more than might be expected from nuclein catabolism. On the other hand, we may contrast with these, as the opposite extreme, the fully adapted terrestrial gastropods—pulmonates such as the English *Helix* and *Helicella* or the Paraguayan *Bulimulus*; or the operculate *Cyclostoma*. Here the retentions were found to be extremely high, ranging from N. 150, W.B. 4 in the case of *Helicella* to N. 700, W.B. 10 in the case of *Helix pomatia* and N. 1000, W.B. 80 in the case of *Cyclostoma*. For these figures it seems quite impossible that nuclein catabolism could be responsible, even if no excretion of uric acid ever took place throughout the organism's life cycle; uricotelic metabolism must be present. It is sometimes supposed that these terrestrial gastropods made their way on to the land by direct littoral penetration; if so, the modern series of periwinkles shows the process in action. Comparative estimations of uric acid in the Littorinidae, inserted on the left of the diagram, show that, whereas the most marine of them (*Littorina littorea*) occupies a position quite analogous to the marine operculates (N. 1.5, W.B. 0.1), the most terrestrial of them (*L. neritoides*) approaches the terrestrial level (W.B. 2.4).

So far, the situation is relatively clear-cut. We now come to the cases which bear on the problem of evolutionary reversibility. First of all there is the interesting marine pulmonate *Onchidella celtica*, a shell-less form which is generally regarded as having made a secondary return to the sea (cf. Joyeux-Laffuie, 1882). It proved to contain almost no uric acid (W.B. 0.27), in contradistinction to the amounts from ten to one hundred times as large found in the terrestrial species. Must we not see in *Onchidella*, then, a far-reaching if not complete abandonment of the uricotelic mechanism, and a return to the excretion of ammonia?

Next come the gastropods of fresh water. Here both the pulmonates and the operculates offer difficulties. Some of the pulmonates, such as *Limnaea stagnalis*, always regarded as secondarily inhabiting fresh water, agree closely with the land pulmonates (N. 100, W.B. 3), and Baldwin (1935) has shown that the developing embryos of *Limnaea*, like those of *Helix*, make considerable quantities of uric acid. On the other hand, *Planorbis* nephridia have definitely less uric acid than those of any terrestrial pulmonate (N. 41), and the amounts of uric acid in the bodies of *Limnaea peregra* and *Ancylastrum fluviatilis* are down almost as low as the marine operculates considered above (W.B. 0.2, 0.6). It might be argued that we have here a series in which uricotelic metabolism is being abandoned with time in an aquatic environment, just as in the periwinkle series uricotelic metabolism is being acquired with time in a terrestrial environment. Caution must, however, be exercised in drawing such conclusions until a much wider range of fresh-water gastropods shall have been examined, not only as regards uric acid retentions but also as regards nitrogen partition of excreta. It must also be remembered that, whereas the lack of an osmotic gradient in marine invertebrates relative to sea water will encourage no more rapid flow of water through the body than would be expected in terrestrial invertebrates, the large osmotic gradient in fresh-water ones may conduce to the excretion of a hypotonic urine in large amounts and the removal of uric acid from

¹ Abbreviations: N. = nephridium; W.B. = whole body: figures in mg. uric acid per g. dry weight.

the nephridium. Unfortunately little or nothing is known about this in the fresh-water gastropods. All that can be said at the present time is that, whereas *Limnaea* hints at an irreversibility of regressive evolution, *Onchidella* and *Ancylastrum* point in the other direction.

The fresh-water operculates, again, are hard to interpret. *Hydrobia jenkinsi*, it is true, seems a clear case, for this mollusc, according to Ellis (1926), was entirely confined to brackish and sea water till near the end of the nineteenth century. Not until 1893 were the first specimens obtained from an inland situation, but subsequently it became abundant in rivers, streams and canals all over England. In conformity with expectation uric acid was found to be almost wholly absent from this gastropod, so that it resembled the marine operculates such as *Buccinum* or *Gibbula*. On the other hand, *Viviparus* (*Paludina*) and *Bithynia* did not behave in conformity with expectation, since they both contained amounts of uric acid corresponding to some of the fresh-water pulmonates such as *Planorbis* (N. 35, W.B. 5). It is usually held that these animals, by reason of their gills and their lack of mantle vascularization, must have reached their present position by colonization up the rivers. Recourse to an explanation based on high water turnover is here impossible, for, while that would explain a case where uric acid was expected and not found, it cannot explain a case where uric acid was found though not expected. Two explanations have been suggested, (a) that uricotelic metabolism is an adaptation to fresh-water as well as terrestrial life, (b) that these fresh-water operculates originated not from the sea direct, but overland, by way of a period of terrestrial or semi-terrestrial life, analogous to that which *Cyclostoma* and *Littorina neritoides* are now living. The former of these two explanations would base itself on a supposed adaptation to aestivation and summer dryness, for which there is very little evidence, and would throw no light on the question of evolutionary reversibility. The latter would refer to the vestigial gill in *Cyclostoma* and would suggest that *Viviparus* and *Bithynia* resembled *Limnaea stagnalis* in retaining the uricotelic metabolism which characterized their earlier days on land. It is possible that the ovoviviparous condition may here be a clue, for the two most terrestrial species of periwinkle (*Littorina rudis* and *neritoides*) are ovoviviparous while the others are not, and *Paludina* certainly is (cf. Lebour, 1935). Ovoviviparity, uricotelic metabolism and terrestrial life would then be associated, in striking contrast to the position of true viviparity and uricotelic metabolism as opposite choices before the pre-reptiles (see Fig. 2). Certain profound differences between these evolutionary situations should, however, be borne in mind. Thus true viviparity, abundant water intake by the maternal alimentary tract, and urea production by the ornithine cycle go together; whereas the only alternative to uricotelic metabolism for the gastropod is ammonia production with its concomitant demand for acid to neutralize it during excretion. It is now fairly certain (Baldwin & Needham, 1934) that the ornithine cycle does not exist in gastropods and that the urea which they produce is entirely derived from the arginine of their diet by way of the arginase system. But ovoviviparity seems very erratic in its distribution, being absent in typically terrestrial and uricotelic gastropods such as *Helix* and *Helicella*.

In sum, there appear to be indications that uricotelic metabolism may, after once having been gained as an adaptation to terrestrial oviparous life, be lost again if a return to aquatic life occurs; but that this loss may be very slow in taking place.

It is pertinent to enquire in this connexion how far the eggs of terrestrial gastropods are cleidoic. Not much is known about them. The observation of Baldwin (1935), that *Limnaea* eggs accumulate uric acid, has already been mentioned, and it has also been shown (Needham, 1935) that a comparison may be made between the eggs of *Helix* and of the hen. The former accumulate 0.3 mg. per cent of the final dry weight in uric acid by the time of hatching; the latter some 0.7 per cent—values of the same order. The snail embryo loses 19 per cent of the dry weight of the egg by combustion; the chick embryo 18 per cent. On the other hand, the snail embryo loses 48 per cent of the wet weight of the egg by evaporation as against the 15 per cent similar loss by the chick embryo. But Carmichael & Rivers (1932) have shown that the snail embryo will still develop normally after a dehydration of the eggs amounting to 85 per cent. There is certainly no evidence that the snail egg absorbs water or anything else but oxygen from the environment, and it may therefore be that the snail, instead of providing the relatively impermeable shell of the bird's egg, provides a much greater excess of water in its eggs above the embryo's minimum requirements.

Finally, it would be of great interest to examine the metabolism of nudibranchs, and tectibranchs such as *Aplysia*, *Philine* and *Oscanius* (about which conflicting reports exist in the literature), also an aspidobranch such as the English *Theodoxus*, or a mollusc such as *Ampullaria*, where the gill and mantle cavity are said to be equally functional. The cephalopods should also receive an extended study, but perhaps most important of all from the point of view of reversibility would be the comparative investigation of the metabolism of some modern reptiles which have a succession of passages between aquatic and terrestrial habit behind them. The investigation of the land crabs would also be profitable with a view to tracing whether uricotelic metabolism has ever established itself in this class also.

V. THE WATER METABOLISM OF CLEIDOIC EGGS

The preceding cases have illustrated the loss of qualities. We have now to see whether the most important part of Dollo's Law, namely, the impossibility of regaining the lost qualities, may be given physiological illustration.

What has already been said about the water metabolism of the cleidoic egg has indicated that the supply of water for the embryos of terrestrial organisms may be for them a very important problem. In a discussion of the evolution of the terrestrial vertebrates, Gray (1928) pointed out that the embryos of all aquatic organisms depend on their environment for a supply of water for the building up of their tissues, and that the egg-white of reptiles and birds is the equivalent of the jelly surrounding amphibian and dipnoan eggs (see also Needham, 1931, § 6). The cleidoic egg was an obvious device to prevent undue evaporation of water. Furthermore, it included means whereby the pressure head of water for the embryo is kept at a relatively constant level, for, as Vladimirov (1926) showed, an as yet unidentified

fixed acid is introduced by the embryonic metabolism into the egg-white, bringing the latter gradually to its isoelectric point and liberating water by degrees from the colloidal albumen.

The margin of safety as regards water loss in the bird's egg is, however, small. On the one hand, it is true that in Weldon's experiments (1902) the addition of water to the incubating egg, in such a manner as not to interfere with the processes

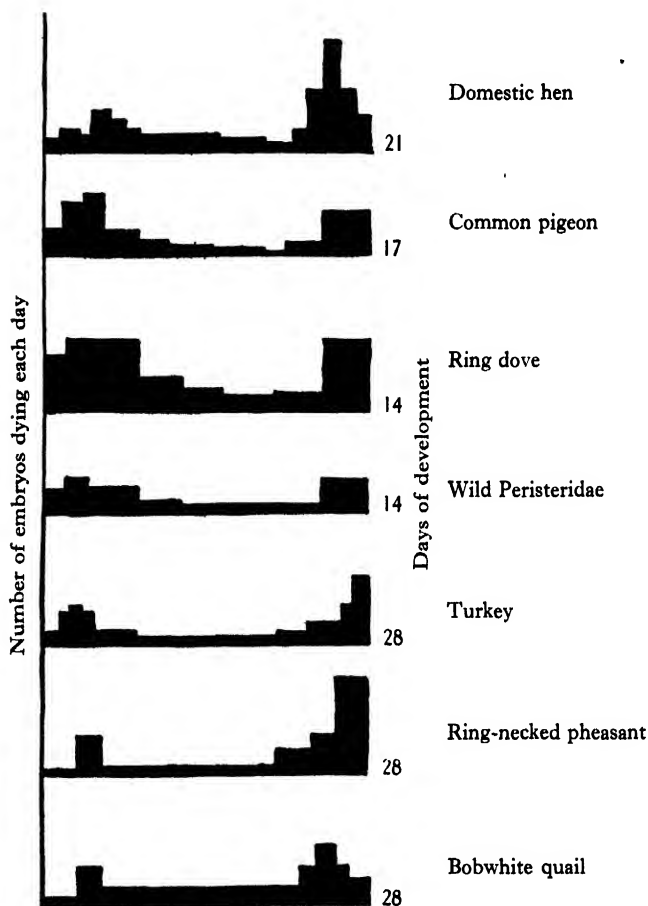


Fig. 4. Distribution polygons showing normal mortalities of seven kinds of birds, the first four from Riddle (1930), the fifth from Martin & Insko (1935) and the last two from Romanov (1934).

of respiration while replacing that normally lost by evaporation, led to malformations of the amnion and the death of the embryo. But, on the other hand, Riddle (1930) has conclusively shown that a small increase of the normal loss of water by evaporation leads also to the death of the embryo, and that the second peak in the normal mortality curve, which occurs around the eighteenth day of incubation, is in all probability associated with insufficiency of water for continued life and development. These peaks are illustrated in Fig. 4, which shows the mortality distribution of the fowl (*Gallus*) embryo compared with that of the embryos of the common

pigeon (*Columba*), the ring dove (*Streptopelia*) and various wild Peristeridae (*Turtur*, *Spilopelia* and *Zenaidura*). In all cases the second peak occurs at an equivalent point of incubation. It is explained by observations on tropical birds. Riddle studied the eggs of pigeons from the climate of Venezuela (the Orinoco region) and found it impossible to hatch them in the temperate, moderately dry air of North America, although if placed partly in water they would develop normally and hatch. Death in the former circumstances usually took place during the final 2 days of incubation and was accompanied by very large air-sacs, indicating excessive evaporation. Indeed, in spite of Weldon's results, Lippincott & de Puy (1923) could hatch hen's eggs successfully if they were incubated lying in a layer of distilled water $\frac{1}{2}$ in deep. Calculation shows that one hen's egg loses by evaporation 9.8 g. water out of an original 38.4 g., and gains from the combustion of fat 2.0 g. This quota may be of importance if the margin of safety is a narrow one, and may be compared with what has been said above concerning the preferential utilization of fat as source of energy by terrestrial embryos.

The case of the Venezuelan pigeons forms one extreme. The other may perhaps be found in the observations of Buxton (1923) on the desert sandgrouse (*Pterocles*), which during the incubation of the eggs saturates its breast feathers with water. This may be important before hatching as well as afterwards.

It is clear that the cleidoic egg of birds is a nicely adjusted mechanism. But the normal mortality curve bears witness that the adjustment is not perfect. And the whole mechanism involves a dilemma, since too thick and impermeable a shell will involve respiratory difficulties in the early stages (first mortality peak) while too thin a one will involve danger of water starvation in the late stages (second mortality peak).

The bearing of this upon the problem of evolutionary reversibility now appears. In view of the urgent need of the cleidoic embryo for water, it might be thought that birds which habitually allowed their eggs to develop in very wet situations, such as the water birds in general, would take advantage of the proximity of this necessary tissue material. But this is far from being the case. Loisel (1900), studying the eggs of the grebe *Podiceps cristatus* and the duck *Anas*, found that when placed in distilled water they absorbed not a trace of it, and gave out no chloride to it over a period of many hours. Their shells were in fact impregnated with some wax or fat, efficiently insulating the egg against the intake of water so valuable for the embryo.

Here then, it seems that cleidoicity is irreversible, and that, once the insulation of the embryo from the environment has taken place, this cannot easily be reversed.

VI. THE GLOMERULUS IN TELEOSTEAN FISHES

In a preceding section we have seen an evolutionary reversibility when elasmobranchs pass from the sea into fresh water. Evolutionary irreversibility appears when we observe the passage of teleosts from the sea into fresh water.

From the early work of Huot (1897) on the morphology of the kidney in the Syngnathidae, a number of investigators have demonstrated that many fishes possess

nearly or completely aglomerular kidneys (Marshall & Grafflin, 1928; Marshall, 1929; Nash, 1931), and in 1930 Smith suggested that the presence of glomeruli was a primitive character connected with the excretion of water, their absence or degeneration in marine teleosts being a specialization due to the oliguria (decreased water excretion) universal among these animals. The suggestion was extended as a theory of evolution of the vertebrate kidney in the brilliant paper of Marshall & Smith (1930).

According to this theory the protovertebrate kidney was primitively aglomerular. It was no more than a series of tubes connecting the coelom with the exterior through the body wall. The glomerulus was evolved in some early Palaeozoic fresh-water chordate to enable the organism to excrete easily the large amount of water entering the body owing to the favourable osmotic gradient between fresh water and animal tissues. The glomerulus was simply "an advantageous juxtaposition of the blood-vascular system to the already existing tubular system draining the coelom." This interpretation connected on the one hand with the conception of the glomerulus as a filter, and on the other with the belief in the fresh-water origin of vertebrates.

Next, with the secondary assumption of a marine habitat, where the osmotic gradient was reversed, or the beginnings of terrestrial life, where water conservation became of the first importance, this glomerular mechanism had to be discarded or amended. Various measures were accordingly taken: (a) the birds replaced the ventral capillaries of their glomeruli with dense syncytium, thus reducing their effective filtration area, (b) the glomeruli of reptiles were invaded by connective tissue, (c) the mammals, by further elaboration of the tubular portion and the addition of the loop of Henle, inserted on the distal side an effective water-absorbing mechanism, parallel with the rectal glands of insects; finally, (d) the marine teleosts abolished the glomeruli altogether in some cases and greatly reduced their number and size in others. Thus in the Hoplodoci and Pediculati (*Opsanus*, *Batrachus*, *Lophius* and *Histrio*) the glomeruli are entirely absent.

Now it might be expected that a return to fresh-water would result in the re-appearance of the glomeruli. But the fact is that it does not. In the fresh-water pipe-fish *Microphis boaja* the aglomerular condition persists, "indicating", as Smith puts it (1932), "that the structure of the kidney is a true phylogenetic character and not a transient adaptation", for *M. boaja* is a derivative of the typically marine Syngnathidae, and must be considered to have secondarily invaded fresh water. Once gone the glomeruli cannot be regained.

On the other hand *Anguilla*, the fresh-water habitat of which is sometimes held to be relatively recent owing to its yearly migration in its modern form, possesses the typical fresh-water development of the glomeruli.

The elasmobranchs offer no special difficulties to the above theory. Since, on entering the sea, they maintained intact their previous favourable osmotic gradient, there would be no reason why they should suppress water excretion through the kidney to any great extent or abolish their glomeruli. And in accordance with expectation, as the review of Marshall (1934) shows, the elasmobranch kidney is glomerular and does not function with so severe an oliguria as that of the marine

teleost (e.g. up to 4 c.c./kg./hr. glom. filtrate in *Squalus acanthias* as opposed to 0.1-0.9 c.c./kg./hr. in the marine teleost *Myoxocephalus octodecimspinosus* and 10-17 c.c./kg./hr. in the fresh-water teleost *Ameiurus nebulosus*). On the other hand, the elasmobranch nephron contains a special segment unknown anywhere else in the animal kingdom. The brush border segment (\equiv *segment à brosse* \equiv proximal convoluted tubule) is intercalated between two segments, identical in structure, which no other vertebrate kidneys show. This structure has been described by Borcea (1906) and, although its function is unknown, it is surmised that it may be connected with urea conservation. Clarke & Smith (1932) have shown that in the dog-fish the filtered urea may be almost quantitatively reabsorbed by the tubule, although a related molecule, such as thio-urea, is not absorbed to any marked extent.

VII. CONCLUSION

In the preceding examples we have seen that on the whole the conclusions of the palaeontologists are supported by the relatively few but remarkable facts of biochemistry and physiology. The final question must now be asked, what is the meaning and explanation of evolutionary irreversibility?

It appears that Dollo himself did not go much further than a vague appeal to the "indestructibility of the past". But "in the last analysis", he said (1913), "it is, like other natural laws, a question of probability. Evolution is a summation of determined individual variations in a determined order. For it to be reversible, there would have to be as many causes, acting in the inverse sense, as those which brought about the individual variations which were the source of the prior transformations and their fixation. Such circumstances are too complex for us to suppose that they ever exist." Essentially, this explanation has something in common with the second law of thermodynamics. The universe is always passing from less probable to more probable states (Royce, 1914). Free energy tends always to decrease and entropy to increase. "Whenever anything happens", says Eddington (1928), "which cannot be undone, it is always reducible to the introduction of a random element analogous to that introduced by shuffling. Shuffling is the only thing which nature cannot undo."

The brilliant discussions of Lotka (1924, 1925) and a more recent essay by Blum (1935) elaborated this position, defining evolution as the history of a system undergoing irreversible changes, and identifying it with the second law of thermodynamics. So also, quite independently, did Blagoveschenski (1929) in his stimulating survey of plant species from the point of view of the organic chemist. Starting from the distribution of alkaloids in the plant kingdom, he concluded that juvenile species have a mainly aliphatic metabolism, and that cyclization, alkaloid formation, etc., may be regarded as a sign of senescent species. He was satisfied that the process cannot be reversed. This "physiological hysteresis" means, according to Blagoveschenski, that the most probable systems are those in which most cyclization goes on, and the final extinction of plant species occurs by a process of self-poisoning. Although Blagoveschenski's views have been accepted by many writers (e.g.

Bertalanffy, 1933), only a plant biochemist could profitably criticize them, and there is an urgent need for their examination, side by side with the remarkable Australian work on the distribution of the essential oil compounds in the eucalypts (Baker & Smith, 1920) and the formation of cyclic compounds from carbohydrates by moulds (Raistrick *et al.* 1931; Birkinshaw,¹ 1937).

The difficulty which we may feel, with Bertalanffy, in a plain correlation between organic evolution and the second law of thermodynamics consists in the fact that the former involves an increase, and the latter a decrease, of organization. In ontogeny it is impossible to believe that a positive increase of organization does not go on as the higher morphological levels come into being, even though in another sense there is a passage from instability to stability. In phylogeny the excessive insistence by the thinkers of the nineteenth century on the view of evolution as a *progress*, caused a reaction to the opposite extreme, yet we really cannot deny that the successive phyla of the animal kingdom represent successive levels of increasing complexity and organization. There is thus a sharp contrast between evolution and the second law. It may or may not be resolved by saying that relatively to the universe the increases of organization involved in phylogeny and ontogeny are small, and may be amply paid for elsewhere by degradations of organization. In any case, supposing the appeal to the second law as a direct explanation of Dollo's Law is justified, we still need to know the mechanism whereby arises the difficulty animal species find in retracing their steps.

Here genetics will probably offer more light than thermodynamics.² We know to-day that the vast majority of mutations, in those cases where they have been well studied, are injurious to the organism, even lethal, and frequently involve the loss or the diminution of some organ or structure. Bristles may be reduced in number or size, eye facets may be suppressed, digits abolished, etc. "Evolution", it has been said, "has proceeded in the teeth of a storm of adverse mutations" (Fisher, 1932). If this is so, and assuming that evolutionary advance has been possible only by the rare favourable mutations, it would seem that by their very rarity (in accordance with Dollo's own hint) it would be improbable that the same favourable adaptation should occur twice, even though the environmental conditions were repeated, as in the secondary or tertiary return to aquatic or terrestrial life. But not only are favourable mutations rare; they are probably also complex. A pattern of more or less simultaneous mutations is probably required in an important favourable adaptation to a new environment. And the more complicated and interlocked this pattern is, the less likely it would be to recur a second time in the shuffling of genes which is always proceeding. The gene-pattern allowing of a new adaptation would thus be formed by chance, and preserved by natural selection once formed, but not likely to be formed again. In this way we may envisage Dollo's Law as the consequence of the genetic equivalent of the second law of thermodynamics.

The work of Wright (1934) on atavistic polydactylism in guinea-pigs is relevant

¹ *Biological Reviews*.

² My friend, Mr C. H. Waddington, hopes before long to publish a discussion of Dollo's Law from the point of view of genetics; here I shall do no more than indicate the relevance of modern genetical studies to this question.

in this connexion. The guinea-pig normally has four toes on its front limbs and three on the hind ones, although a supernumerary toe is not very uncommon. Wright discovered a dominant gene for toe reduplication, which gave, in the heterozygous condition, as many as five toes on both limbs, thus resembling the earlier pentadactylous ancestors of this species. In the homozygous condition the gene was lethal, but gave an abnormally large number of toes to the embryos before they died. It may be concluded that change in toe number without serious concomitant derangements took place by the interaction of several genes, and that by crossing, this balance may be upset. Left to themselves, the guinea-pigs would never have stabilized the atavistic condition; Wright was only able to do so by careful selection of the necessary modifying genes.

VIII. SUMMARY

1. It has been the object of this article to draw attention to biochemical and physiological evidence which supports the generalization known as Dollo's Law, side by side with palaeontological and morphological evidence.
2. Evolution is reversible in the sense that structures or functions once gained can be lost, but irreversible in the sense that once lost they can never be regained. Loss of properties is illustrated, (a) by the enzymic equipment of bacteria and Protozoa, (b) by the relative uraemia of marine and fresh-water elasmobranch fishes, (c) by the uricotelism of terrestrial and fresh-water molluscs. Failure to regain properties once lost is illustrated, (a) by the cleidoicity of the eggs of aquatic birds, (b) by the glomerular status of the kidneys of marine and fresh-water teleostean fishes.
3. Why there should be this irreversibility in evolution is a problem of fundamental interest. If the second law of thermodynamics is responsible, we may ponder the curious paradox that while on the one hand there is in evolution an increase of organization as ever higher groups and phyla appear, there is also a diminution of organization associated with the shuffling process of entropy increase—the only thing nature can never undo.

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CROSSING-OVER

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I. INTRODUCTION

CROSSING-OVER, or the interchange of segments between homologous chromosomes, is usually considered in its relation to the recombination of genetical factors. Through this connexion its study, as undertaken by Morgan, Sturtevant, Bridges, Muller and others of the *Drosophila* school, has, in the past, provided the means of analysis of the organization of linkage groups. This work is fundamental to the chromosome theory of heredity as we know it to-day, but an account of it would be superfluous here as the results are both well known and set out in considerable detail elsewhere (cf. Morgan *et al.* 1925; Stern, 1931 *a*). Furthermore, the study of recombination has elucidated a number of points relating to the mechanism of crossing-over itself, as, for example, the property of interference between successive cross-overs (Muller, 1916).

It has, however, long been known that crossing-over may be expressed in other ways. Indeed, it was by the finding of exceptional recessive segregates that Bridges, in 1916, was led to the conclusion that crossing-over occurred in the "four strand" stage. In general it is clear that crossing-over, being a property of the chromosomes, may be observed in a number of different ways, either cytological, by direct observation of the chromosomes, or genetical, by observing the inheritance of alternative

dominant and recessive allelomorphs, which have been used to mark alternative homologous chromosomes.

Of the various expressions of this phenomenon of crossing-over, each shows its peculiar relations to the underlying cause, and each contributes different information as to the mechanism whereby crossing-over is brought about.

It was not possible fully to co-ordinate the different kinds of evidence before the demonstration of the chiasmotype theory. Now that this theory, stating that crossing-over is the sole agent conditioning the formation of the cytologically visible chiasma, is known to be true, a new and powerful method of analysis is to hand, viz. the joint use of genetical and cytological data. The value of this approach is partly due to the increased range of organisms open to observation and partly due to the complementary nature of the two kinds of information. The genetical method allows of the study of portions of individual chromosomes, but is subject to the limitations of the necessity for the use of mutant genes and of the lapse of time between the act of crossing-over and the observation of its effect in a later generation. The cytological method allows of the immediate observation of the effects of crossing-over without the necessity for the use of mutants; but, save in a few cases, it does not allow of the study of individual sections of a chromosome or even of individual chromosomes. The chiasmotype theory in combining these two sets of observations is fundamental to the present day approach to the problems of crossing-over.

It is the object of this review to present the main aggregates of evidence relating to crossing-over, its determination and expression. These two aspects of the question are clearly closely related but may be formally separated for the purpose of presentation. As the behaviour of any given expression cannot be considered without knowledge of the potentialities and limitations of crossing-over itself, the determination of crossing-over will be considered first and then attention will be turned to the modes of expression of this phenomenon. In dealing with the latter, the discussion will be confined to the relations between crossing-over and its various expressions. The use in research, as for example in the analysis of linkage groups, to which the effects of crossing-over have been put is beyond the scope of this article.

II. THE DETERMINATION OF CROSSING-OVER

Cytological evidence (Rückert, 1892; Jannsens, 1909, 1924; cf. Darlington, 1936 *c*) shows that the first division of meiosis is the only division at which the homologous chromosomes regularly exchange partners. Genetical evidence from tetrad analysis of the four spores produced by a meiotic division (Allen, 1926; Wettstein, 1924; Dodge, 1929; Lindegren, 1932; Goldschmidt, 1932; Brieger, 1933; Mather, 1935 *a*) confirms this in that it shows segregation as an immediate consequence of reduction division. This evidence, however, leaves open the question of when during meiosis the crossing-over occurs.

A further question is that of where, in the chromosomes, exchange occurs. A study of cytologically visible exchanges of partner indicates that in some cases at least, e.g. *Mecostethus* sp. (McClung, 1927; White, 1936) and *Fritillaria meleagris*

(Newton & Darlington, 1930), exchange is confined to certain restricted sections of the chromosomes. Are these special cases or is some localization of exchange the general rule? These questions of when and where crossing-over occurs must be answered before it is possible to consider how it occurs.

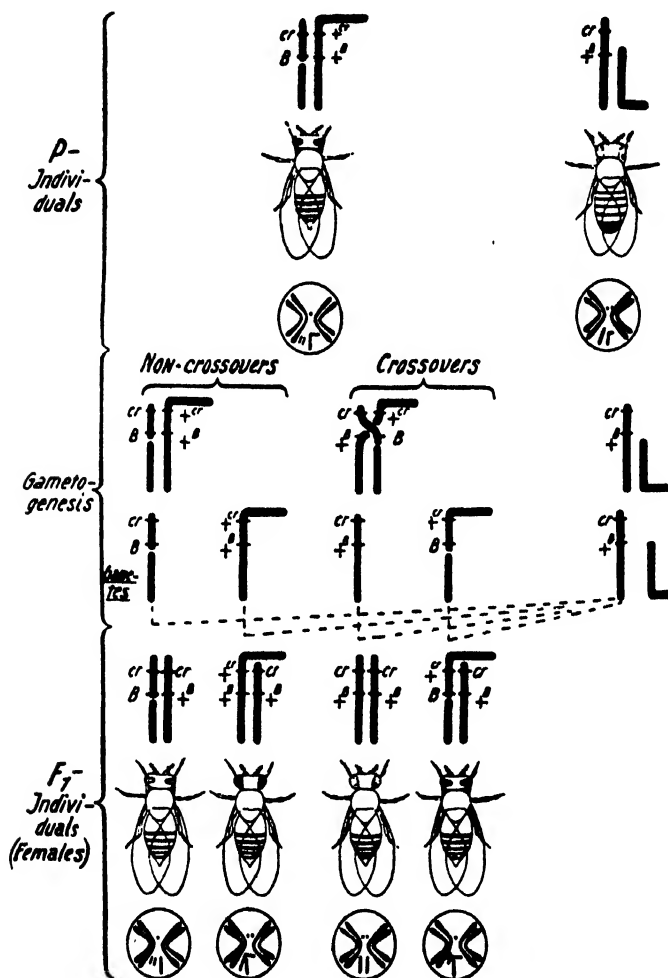


Fig. 1. Diagram illustrating the coincidence of genetical and cytologically detectable crossing-over in *Drosophila*. Top left: female heterozygous for carnation and Bar, together with her chromosomes. Top right: carnation not-Bar male and his chromosomes. Below: the chromosomes and genetical phenotypes of the female offspring of the above flies, produced both with and without crossing-over, assuming that genetical and cytological crossing-over are the same. These, and only these, types were commonly found in experiment (Stern, 1931 *b*, after Darlington, 1936 *c*).

(1) *The coincidence of genetical and cytological crossing-over*

Before proceeding with the above consideration it is necessary to point out that full proof of the coincidence of genetical and cytological observable crossing-over is provided by certain experiments of Stern (1931 *b*), Creighton & McClintock (1931) and Brink & Cooper (1935). These all consist of marking the homologous chromo-

somes with both genetical and cytological characters. The former are genes and the latter cytological visible changes in chromosome structure. The progeny from such individuals may then be classified in two ways according to the genetically detectable crossing-over between gene loci and the cytologically detectable crossing-over between visible chromosome changes. In every case the two classifications agreed within the limits of error expected for the experiment (Fig. 1). We may then conclude that the two methods are but technically different in that they allow of the study of the same structures by alternative approaches. Their results may be combined in one analysis.

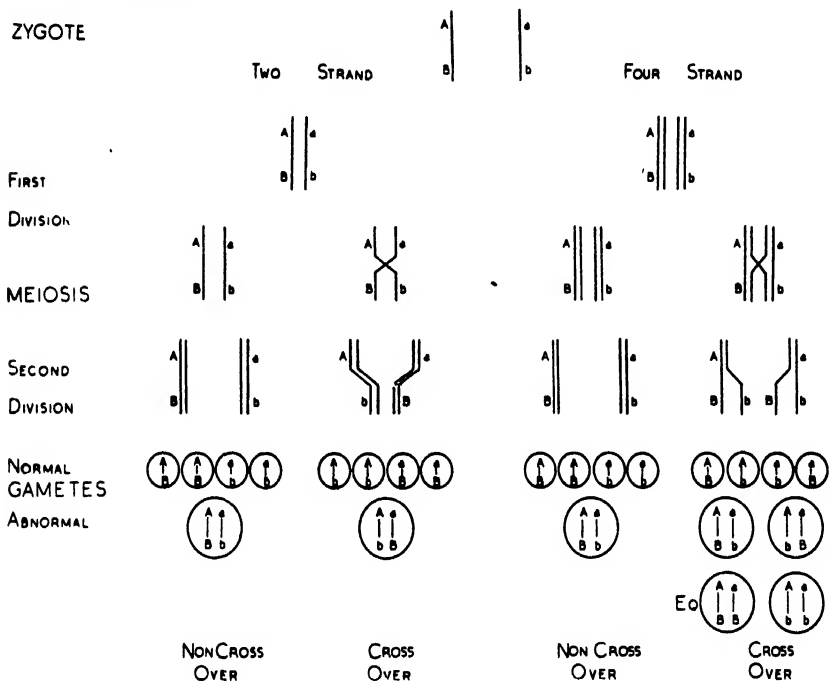


Fig. 2. Bridges's demonstration of four-strand crossing-over. On the left: crossing-over and the formation of normal (monosomic) and exceptional abnormal (disomic) gametes when crossing-over occurs in the two-strand stage. On the right: the same but with four-strand crossing-over. In the latter case, but not in the former, certain equational exceptions (*EQ*) are produced and so when found demonstrate four-strand crossing-over (Bridges, 1916). In this diagram failure of separation is assumed to occur at first meiotic division and the two chromatids associated at second division must pass to different gametes at second anaphase. A similar set of equational exceptions for *A*, *a* could be produced.

(2) The time of crossing-over

Genetically it has been shown in *Drosophila* (Bridges, 1916; Bridges & Anderson, 1925; Anderson, 1925; Redfield, 1930, 1932; Emerson & Beadle, 1933; Beadle & Emerson, 1935), *Zea* (Rhoades, 1933) and *Habrobracon* (Whiting & Gilmore, 1932), that crossing-over must occur at the "four-strand" stage, i.e. after the homologous chromosomes have each divided into two chromatids. These demonstrations depend on obtaining disomic gametes with those two homologous chromosomes, which replace the usual single member, different in respect of crossing-over. One is a cross-

over and the other a non-cross-over, the crossing-over being detected by the use of genes.

Now crossing-over between two single chromosomes would give two complementary cross-over chromosomes. So in order to obtain in a single gamete two unlike and non-complementary chromosomes from the two homologues, as for example a cross-over and a non-cross-over, it is necessary to postulate that *each of the homologous chromosomes is double at the time of crossing-over and that but one of the two halves of each takes part in crossing-over* (see Fig. 2). This is crossing-over in the four-strand stage. In the case of triploids the corresponding stage would, of course, be six-strand.

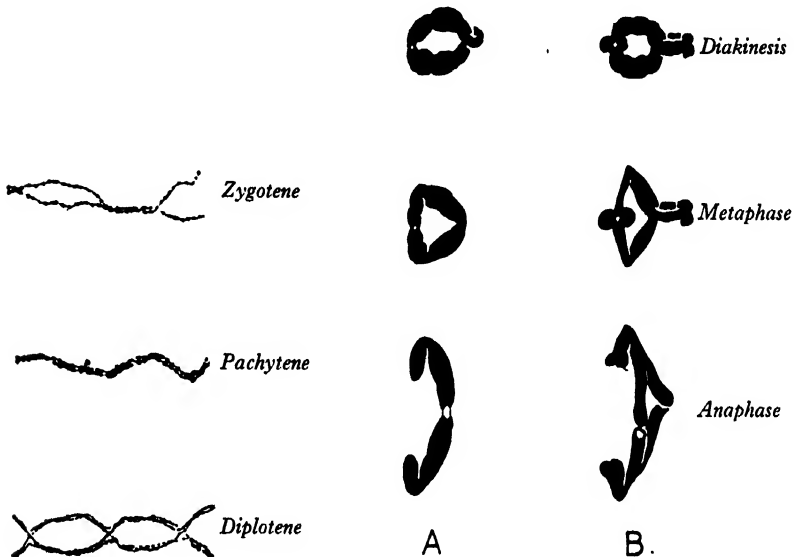


Fig. 3. The meiotic behaviour of a bivalent (A) with terminalization of the chiasmata and (B) without terminalization. Note the production of the cross-shaped chiasmata when the chromosomes become double and fall apart at the end of pachytene.

These observations limit the stage of first meiotic division at which crossing-over occurs to the period between the end of pachytene, when the homologous chromosomes are fully associated in pairs but individually still single, and first anaphase when the homologous chromosomes cease to show intimate contact with each other, and separate to opposite poles.

Cytological evidence is necessary in order to go further. At the end of the pachytene stage the paired chromosomes divide to give quadripartite structures which open out into loops. The two sides of each loop are composed of paired half chromosomes, or, as they are called, chromatids, and the junction of two loops in the same bivalent is marked by a cross-shaped exchange of partner, the "chiasma" (Fig. 3). This is the diplotene stage. The bivalents then contract and the number of chiasmata may be reduced. The chiasmata also, in some cases, move to the ends of the chromosomes (Darlington, 1929*a*, 1936*c*). It is clear that these partner exchanges or chiasmata must be concerned with crossing-over. They represent the points of

contact between the homologous chromosomes and also show, in any interpretation, the two halves or chromatids of each chromosome to be behaving differently from one another, thus agreeing with the genetical requirements.

The chiasmata may be related to crossing-over in two ways. The first possibility is that chiasmata are formed as a direct result of crossing-over between the homologous chromosomes (Belling, 1931, 1933; Darlington, 1930 *b*, 1931 *a*). Then identical or sister chromatids, i.e. those derived from the same chromosome, are associated on each side of the chiasma (Fig. 4 *a*) unless, of course, the chiasmata have moved their positions. On this view, as chiasmata appear as soon as the chromosomes divide, crossing-over must occur practically simultaneously with this division of each chromosome into two chromatids.

The second interpretation of the nature of chiasmata is that they form as a result of the opening out of the four chromatids along two different planes in different regions. Then where the different partitions meet, there must result an exchange of partner. This is, of course, a chiasma. Crossing-over occurs when chiasmata are resolved by breakage and rejoining (Fig. 4 *a*). This was the view supported by Wenrich (1916) and Sax (1930, 1932).

Before proceeding to a detailed consideration of these alternatives it may be noted that they supply different interpretations of the reduction in the number of chiasmata between diplotene and metaphase. The former view demands that such reduction be due to a fusion of the chiasmata, presumably at the ends of the bivalents, in the process of terminalization. This receives some support from various statistical data on chiasma frequencies and is able to account for the reduction completely (Darlington, 1936 *c*). The second view holds that chiasmata are lost by resolution and resulting crossing-over. Thus the chiasmatype theory has survival of chiasmata related directly to crossing-over. The so-called classical theory relates loss of chiasmata to crossing-over.

Various critical configurations allow of a decision being reached between the rival theories. The arguments on which the conclusions are based are variously developmental, functional, and physiological.

Perhaps the most satisfactory demonstration of the relation between crossing-over and chiasma formation is provided by the case of double interlocking (Mather, 1933 *b*). Interlocking between bivalents is dependent on the chromosomes becoming entangled during pairing at the time when they are still single. One or both of a pair may become imprisoned between the two members of a second pair (Gelei, 1921; Levan, 1933). If chiasmata are formed by the first pair on both sides of the point of imprisonment the interlocking relationship will be retained until metaphase. If one member of the second pair passes between the first pair the interlocking is "true". Where both members of the second pair are caught it is "false" (Fig. 4 *b*). It is clear from the diplotene and metaphase configuration obtained (Mather, 1935 *d*) that interlocking is always determined *before* the chromosomes divide.

In the critical case of interlocking observed at diakinesis one loop of a bivalent has its two sides passing through two separate but adjacent loops of a second bivalent (Fig. 4 *c*). The interlocking at pachytene, before the occurrence of splitting

and chiasma formation, must have been of the "false" type but with the members of the second, or inner, bivalent held apart by pairing of the first or outer bivalent between them (Fig. 4 *c*). Chiasma formation then occurs in the central paired portion of the first bivalent and various other places. The interpretations of the chromatid structure of these bivalents on the opposed chiasmatype and classical

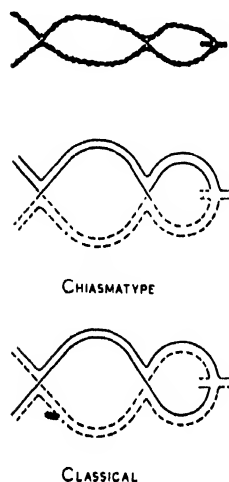
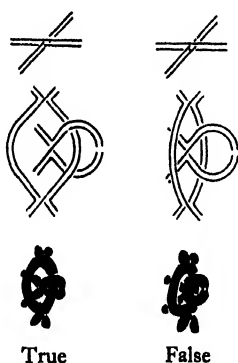
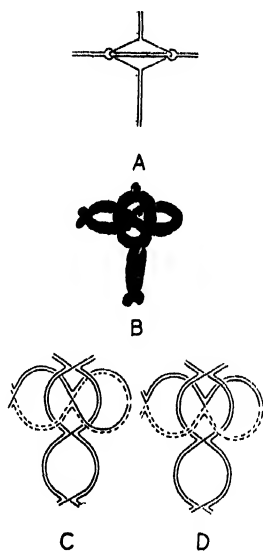
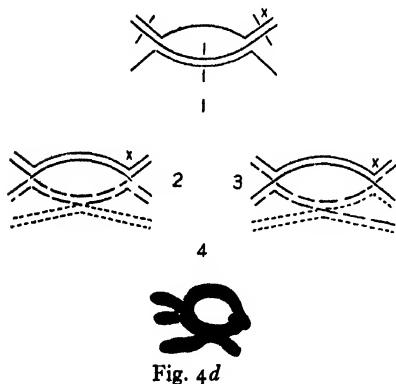
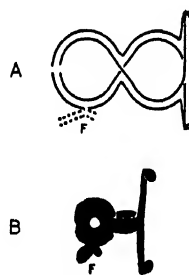
Fig. 4 *a*Fig. 4 *b*Fig. 4 *c*Fig. 4 *d*Fig. 4 *e*

Fig. 4 *a*. Top: a diplotene bivalent with three chiasmata, and, below: the two interpretations of this bivalent on the chiasmatype and classical theories respectively. The chromatids from one chromosome are shown solid and from the other, broken.

Fig. 4 *b*. Interlocking, true and false. Top: the pachytene configurations. Middle: diagrams of the structures after chiasma formation. Bottom: the appearance of the actual configurations at diakinesis. Fig. 4 *c*. The critical case of double interlocking. A. The pachytene association. B. The configuration observable at the later prophase stages as seen from the side. C. The classical interpretation showing sister chromatids on opposite sides of the intruded chromosome in one of the two loops. D. The chiasmatype interpretation. C is incompatible with A (Mather, 1933 *b*).

Fig. 4 *d*. Trivalent formation. 1, the pachytene association with two chromosomes associated and one unassociated at any level; 4, the resulting diakinesis configuration of which 2 and 3 are the rival interpretations. X marks the critical chiasma. The classical interpretation, 3, is incompatible with 1. Fig. 4 *e*. Pairing of a fragment (*F*) and large chromosome. A is the chiasmatype interpretation of the configuration B, observable at meiotic metaphase. No classical interpretation is possible.

theories, are shown in Fig. 4 *c*. The former interpretation is in complete accord with what is known of the development of interlocking. The classical view has, however, identical or sister chromatids on opposite sides of the intruded arm of the second bivalent in one loop of the first bivalent. This cannot be so, as the interlocking was determined *before* the chromosomes split, i.e. before the identical chromatids separated. Thus the classical view is contradicted by the observed configuration. The evidence is in favour of the chiasmotype theory which holds that chiasmata are the result of crossing-over. The classical view could, however, be reconciled with the configuration if it is supposed that two central chiasmata had existed and that one broke and resolved. This is, however, a remote possibility in *Lilium* where the configuration was first observed (Mather, 1935 *d*) and, as will be seen below, becomes untenable in face of accumulated evidence.

Unfortunately, the critical interlocking configuration has been observed but three times in all, twice in *Lilium* sp. (Mather, 1933 *b*; Beal, 1936) and once in *Eremurus* (Upcott, 1936). A much more common configuration, supplying evidence for a decision between the rival theories, is frequently found in polyploids (Darlington, 1930 *b*; Darlington & Mather, 1932).

In polyploid organisms the homologous chromosomes are associated, at pachytene, in pairs, but with changes of partner at various places (Fig. 7). Chiasmata form only between paired portions of chromosomes. The critical case occurs where one chromosome forms a chiasma with a second member between two chiasmata which it has formed with a third. The alternative interpretations are shown in Fig. 4 *d*. The chiasmotype interpretation agrees with the pachytene observations. The classical interpretation has chromatids from three distinct chromosomes associated in the formation of one end chiasma (marked *X* in Fig. 4 *d*). This is in contradiction to the observably strict association in pairs, at any level, at pachytene. This difficulty may be overcome again by postulation of a resolved second chiasma, but correspondingly the evidence is opposed to such a conjecture (cf. Appendix to Darlington & Mather, 1932).

A third source of evidence is provided by the formation of single chiasmata between homologous sections of chromosomes whose ends are distinguishable. The pairing of chromosome fragments in *Lilium henryi* and *L. japonicum* (Mather, 1935 *d*) shows many examples of this. The central part of the fragment forms a single chiasma with a large chromosome. It can be seen that identical chromatids are paired on each side of the chiasma, as the ends of the fragment are distinguishable morphologically from those of the large chromosome (Fig. 4 *e*). The possibility of two chiasmata between the fragment and large chromosome is extremely remote, and so the evidence is again in complete agreement with the chiasmotype theory but contradicts the alternative classical view.

Similar demonstrations to this last one are afforded by unequal bivalents, where, however, only one end of the chromosome is marked by inequality (Catcheside, 1932; Koller, 1932; Mather, 1934).

In *Oenothera* (Darlington, 1931 *b*) and *Pisum* (E. R. Sansome, 1932) the segmentally interchanged chromosomes provide a similar configuration (the "figure-of-

eight") but here the chromosome ends are distinguished physiologically by their pairing properties, and not morphologically as before.

Finally the effects of pairing in homologous chromosomes, one of which has a segment inverted with respect to the centromere, and also the coiling behaviour of the chromosomes at meiosis are phenomena which can be fully explained if the chiasmatype theory is adopted (Darlington, 1935 *a*, 1936 *b*, *c*). The opposite hypothesis leads to difficulties in accounting for the observed results.

In every case the postulation of an imaginary revolved second chiasma would bring the classical view in accordance with observation, but even in individual cases this can be shown to be a remote possibility and over the whole of the evidence it becomes an infinitely remote contingency. The chiasmatype theory is supported without exception.

A combined genetical and cytological study of *Zea-Euchlena* hybrids by Beadle (1932 *a*) supplies further evidence, if it is needed. He observed 12 per cent genetical crossing-over and 20 per cent chiasma formation in one chromosome segment. Now on the chiasmatype theory a mean frequency of formation of one chiasma is equal to 50 per cent genetical crossing-over (50 centimorgans), because two of the four strands cross-over at each chiasma. Hence 12 per cent crossing-over corresponds to 24 per cent chiasma formation. The 20 per cent observed is in fair agreement with this expectation.

Finally we may note that cytologically observed chiasmata and genetically inferred points of crossing-over show parallel properties, as they should on the chiasmatype view. At the risk of some anticipation of later sections examples of this parallelism may be listed here for convenience.

(1) Genetically inferred points of crossing-over and cytological chiasmata have similar statistical properties (Mather, 1933 *a*).

(2) Failure of pairing and non-disjunction is inversely related to crossing-over in *Drosophila* (Detlefsen & Roberts, 1921; Anderson, 1929; Dobzhansky, 1933) and to chiasma formation in nearly all cases studied.

(3) Disjunction is correlated with crossing-over in triploid *Drosophila* (Rhoades, 1933) as it is with chiasma formation in trivalents (cf. Darlington, 1936 *c*).

(4) The incidence of crossing-over and chiasma formation differs between the sexes in mice (Crew & Koller, 1932).

(5) Crossing-over and chiasma formation show the same peculiar properties in male *Drosophila* (Darlington, 1934; Philip, 1935).

(6) Temperature variations affect crossing-over and chiasma formation in the same manner (Plough, 1917; White, 1934; cf. also Mather, 1936 *c*, 1937).

(7) Both crossing-over and chiasma formation show competition, i.e. their frequencies in different bivalents are not independent (Morgan *et al.* 1933; Mather & Lamm, 1935; Mather, 1936 *a*; Steinberg, 1936).

This demonstration of the validity of the chiasmatype theory, i.e. that crossing-over conditions chiasma formation, allows us to state when crossing-over occurs with precision. It must occur practically simultaneously with chromosome splitting at the end of the pachytene stage of meiosis. Furthermore, it involves the two

chromatids of each chromosome unequally, one crossing-over and the other retaining its original state intact.

(3) *Multiple strand crossing-over; interference*

The fact that chiasma formation occurs when the chromosomes are already double leads to a fresh view of the relationships of the strands which cross-over at (a) individual chiasmata, (b) two or more chiasmata.

There are two distinct possibilities with respect to strands crossing-over at any chiasma. These two of the four strands concerned may be derived theoretically either from the same parental chromosome or one from each of the two original chromosomes. The former is known as sister strand crossing-over, and will seldom lead to any recognizable genetical rearrangement. In fact it has needed considerable work to obtain any data whatsoever relative to this phenomenon. Sufficient experiments have, however, now been carried out, particularly in a special type of *D. melanogaster* having a ring-shaped, or closed, *X* chromosome, for it to be said with some confidence that sister strand crossing-over probably never occurs (L. V. Morgan, 1933; Weinstein, 1936). Consequently it will be assumed in the following discussion that crossing-over is always between two non-identical chromatids. This is, of course, the type of crossing-over that leads to cytologically visible chiasmata and to genetically detectable crossing-over.

It has long been known that where multiple crossing-over, i.e. more than one cross-over in the same chromosome, occurs, the various points are not independent of one another. The occurrence of one point of crossing-over in any region of a chromosome lowers the possibility of further crossing-over in its immediate neighbourhood. As the distance from the first point increases, this interference, as it is called, decreases in magnitude until independence is attained (Muller, 1916; Weinstein, 1918). Now it is clear that with "four-strand" crossing-over interference may be of two kinds, a genuine lowering of the probability of chiasma formation in the immediate neighbourhood of a pre-existing chiasma; or, on the other hand, a non-random relationship between those two of the strands which cross-over at the second chiasma with respect to the two crossing-over at the first. These types of interference are known respectively as chiasma and chromatid.

Since chiasma interference is a property of the chiasma as a whole, and not of any constituent chromatid, it is possible to detect its occurrence by a mere study of the distribution of the chiasmata themselves. Haldane (1931) has shown that in the absence of interference the frequency distribution of bivalents with various numbers of chiasmata should be in the form of a Poisson series

$$e^{-m} \left(1, m, \frac{m^2}{2!}, \dots, \frac{m^r}{r!}, \dots \right)$$

where m is the mean. The main feature of the Poisson series is that its variance, obtained from the sum of squares of the deviations of individual points from the mean, is equal in value to the mean itself. In all cases examined it has been shown that this is not so. The variances of chiasma frequency distributions are universally

lower than their means, usually being about one-quarter or one-fifth of this value. This is evidence of chiasma interference. Similar evidence of interference can be obtained from genetical experiments in which the entire length of a chromosome has been marked by a number of mutant genes so allowing of a complete study of crossing-over in the chromosome concerned (Mather, 1933 *a*).

Chromatid interference is much more difficult to detect. As noted above, this type of interference depends on non-random relations of the strands crossing-over at any pair of chiasmata. There are three possible relationships of this kind. In the first place those strands which crossed-over at the first chiasma may again cross-over at the second, giving reciprocal crossing-over. Secondly, those two strands which failed to cross-over at the first chiasma may cross-over at the second, giving complementary crossing-over. The third and final possibility is that one cross-over and one non-cross-over strand from the first chiasma may cross-over at the second,

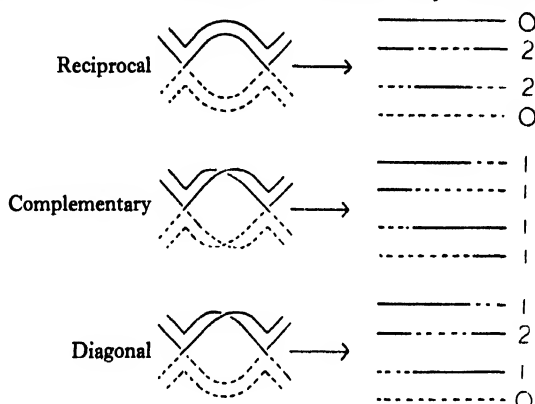


Fig. 5. The three relations of crossing-over at two successive chiasmata. On the right are the chromatids produced by the various possible relations, marked according to the numbers of cross-overs that they contain.

producing diagonal crossing-over (Fig. 5). It will be noticed that the first two kinds of crossing-over result in the chromatid relationships which existed prior to the first chiasma being restored by the second. This is compare crossing-over or, in other words, the chiasmata are compensating. The third relationship does not have this property and the crossing-over is then said to be disparate, or the chiasmata are non-compensating.

As one or other of the two chromatids derived from each chromosome crosses over at each chiasma, any strand will have a half chance of undergoing crossing-over. It then follows that where the first chiasma marks two of the strands by the fact that they have crossed-over at it, the same two strands will both undergo crossing-over at a second chiasma in $(\frac{1}{2})^2$, or $\frac{1}{4}$ of the cases. Complementary crossing-over will similarly occur in a quarter of the cases, the remaining half of the cases showing diagonal crossing-over. Reciprocal crossing-over gives two double and two non-cross-over strands, complementary chiasmata give four single cross-over strands and finally diagonal crossing-over gives one double, two single, and one non-cross-over (Fig. 5). As these relations occur, in the random case, in $\frac{1}{4}$, $\frac{1}{4}$ and $\frac{1}{2}$ of the cases

respectively, the average output of two chiasmata will be $\frac{1}{4}$ double cross-over, $\frac{1}{2}$ single cross-over, and $\frac{1}{4}$ non-cross-over strands. These proportions may be expressed as $(\frac{1}{2} + \frac{1}{2})^2$. With n chiasma the frequencies of n , $n-1$, $n-2$, etc. crossing-over strands are similarly given by the expansion of the binomial expression $(\frac{1}{2} + \frac{1}{2})^n$.

This rule will not hold, however, when the probability of a cross-over strand again crossing-over at the next chiasma is raised or lowered with respect to half, i.e. when the two strands cross-over at the second chiasma are not independent of those crossing-over at the first. This is chromatid interference. The general case where this is true is difficult to consider and indeed no occasion has yet arisen demanding its detailed mathematical analysis. It might, however, be noted that, in the case of two chiasmata, if the probability of a cross-over strand again crossing-over at the second is p , the proportions of double, single, and non-cross-overs will be given by $(p+q)^2$ or $p^2 + 2pq + q^2$ where $q = 1 - p$. Where p is not equal to $\frac{1}{2}$, $2pq$ must always be less than $\frac{1}{2}$. This leads to the interesting conclusion that diagonal or disparate crossing-over should never occur in more than $\frac{1}{2}$ of the cases, unless some very unusual and unexpected circumstances are met with.

Chromatid interference has been detected by both genetical and cytological means. Details of the results will be given in a later section. It is, however, important to notice that whereas chiasma interference appears to be universal in occurrence, strong chromatid interference would appear to be the exception rather than the rule.

(4) *The position of crossing-over*

There is abundant evidence available that in some organisms the points at which crossing-over can occur are localized. In *Mecostethus* sp. (McClung, 1927; White, 1936) and in *Fritillaria meleagris* (Newton & Darlington, 1930) it can be seen that chiasmata always form in close juxtaposition to the spindle attachment or centromere. In both organisms chiasmata may also form in the distal regions, but these are variable in occurrence. Darlington (1935 *b*) has also shown that this localization of chiasmata is not absolute, as some species of *Fritillaria* may show it to a greater or less degree. The question then arises as to whether localization of crossing-over is an exceptional phenomenon confined to some groups of organisms, or is general in occurrence.

Several considerations of a general nature point towards the universal occurrence of localization of chiasmata. In the first place it is a matter of common observation that univalents, i.e. bivalents in which chiasma formation has failed, are distinctly a rarity in normal diploid non-hybrid organisms. With random chiasma formation in the bivalent, or randomness modified by the occurrence of chiasma interference, which clearly cannot affect the first chiasma to form, and a mean of approximately two chiasmata per bivalent, as is quite common, a substantial amount of univalent formation might be expected. Of course it can be argued that the chance of obtaining a chiasma in any small section of a chromosome is very high, but that interference is also very strong. In some ways, however, it seems more plausible to

imagine that some small parts of the bivalent enjoy a high chance of forming a chiasma, and that the number of these parts is low. This is, of course, localization. This latter explanation is supported by the behaviour of true-breeding organisms in which there is a great disparity in size between the members of the chromosome complement, e.g. *Stenobothrus*. This disparity is not reflected in the chiasma frequencies as the small bivalents have a relatively very high frequency of chiasmata. Such a relation between chromosome length and chiasma frequency is explicable as either (a) the chance of crossing-over per unit cytological length increasing in small chromosomes, or (b) the presence of local regions of high crossing-over in all chromosomes including the small ones where, of course, they comprise a larger proportion of the body of the chromosome. The latter explanation seems definitely the more reasonable. Its truth is fully borne out by a more detailed analysis of the chromosome length-chiasma frequency relationship.

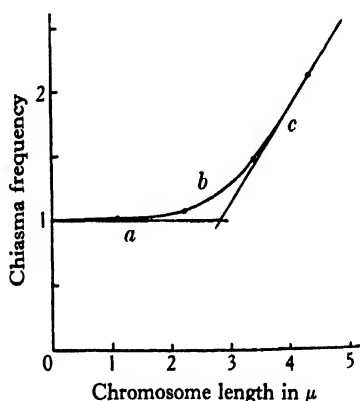


Fig. 6. The chromosome length-mean chiasma frequency relationship. In the region (a) the chiasma frequency is 1 irrespective of chromosome length; (b) is a region of change of relationship and in (c) the chiasma frequency increases with the chromosome length (Mather, 1937).

Where there is a large size disparity among the chromosomes of the complement, the plotting of chiasma frequency against chromosome length gives a curve of the type shown in Fig. 6. There are three distinct regions to this curve, (a) where one chiasma is formed independently of the length of the chromosome, (b) a region of smooth change between the other portions, and (c) where the chiasma frequency increases with the chromosome length (Mather, 1937). The central region of change may be neglected as due to the innate variation in number and position of chiasmata. Attention is then devoted to the regions (a) and (c) of the curve relating mean chiasma frequencies and cytological lengths.

Taking the latter relation first, it may be interpreted as each chiasma occupying, on an average, a piece of chromosome practically to the exclusion of others. This is clearly interference in a different guise. When a chiasma is formed it suppresses further chiasma formation in its vicinity and so "occupies" that section of the chromosome or bivalent. To achieve an increase in the mean chiasma frequency an extension of the chromosome of length sufficient to accommodate the extra chias-

mata is necessary. The fact that this region of the curve is a straight line shows that the interference properties of the chiasmata are constant or very nearly so within and between the bivalents of the complement (cf. Mather, 1937, on data from Levan, 1933, 1935, and Stone & Mather, 1932). With widely varying interference relations in different bivalents or even parts of the same bivalent, this region would be curved.

The first portion (*a*) of the chiasma frequency-chromosome length curve is of a nature differing considerably from (*c*). It shows the bivalents to be forming one chiasma irrespective of their length. As this section of the curve concerns the formation of one chiasma only, there can be no question of interference entering into the consideration. The extent of region (*a*) is apparently limited to some maximum length beyond which the chromosome has a mean chiasma frequency of more than one. We may use the same method of interpretation as for the region (*c*) and say that the first chiasma occupies a characteristic length of the chromosome, but that this length varies with the total chromosome length. Below a certain chromosome size the length occupied by the first chiasma comprises the whole of the chromosome, but above this size the first chiasma occupies a certain length, the rest being available for the formation of further chiasmata.

We are now in a position to give a complete description of the behaviour of the long chromosomes. A first chiasma is formed in, and occupies a characteristic length of bivalent; this length varies between the bivalents. If the chromosome is sufficiently long further sections of the bivalent may be occupied by additional chiasmata, each taking up a length which is constant within the nucleus. The length appertaining to the first chiasma is the *Differential Distance* and that of subsequent chiasmata is the *Interference Distance* (Mather, 1937).

The relation between chromosome length and chiasma frequency may thus be written

$$y = 1 + \frac{x-d}{i},$$

where y = mean chiasma frequency, x = chromosome length, d = differential distance, and i = interference distance. d and x are variable but i is constant.

Further analysis is possible. It is clear that in general d does not equal i . Therefore d , unlike i , is not a mean distance between two chiasmata. It is, then, most likely the mean distance between the first-formed chiasma and some point or landmark of the chromosome. Hence the positions of the chiasmata are determinate relative to some point of the chromosome. This will most likely be a fixed point, e.g. the centromere, or the end of the chromosome, in which case the frequency of crossing-over is not constant per unit cytological distance within a bivalent, i.e. there is always some degree of localization.

The truth of this interpretation of the length chiasma frequency relation may be tested by various experimental data. As d and i are not immediately related in magnitude it should be possible, theoretically, to vary them entirely, or partially, independently. A change of d independently of i will give parallel sloping parts to the curve. A change of i independently of d will result in pencils of sloping lines. This is found to be the case.

White (1934), using three species of orthopteran insects, *Locusta migratoria*, *Schistocerca gregaria*, and *Stenobothrus parallelus*, estimated the chiasma frequencies over a range of temperatures. Some of his results are shown in Fig. 7 a. Change resulting from temperature alteration is one of position, but not slope, of the lines. It is a change in the differential distance unaccompanied by a change in the interference distance.

Moffet (1936) finds the opposite effect. He counted the chiasmata of different individuals of *Culex pipiens*, which have, unfortunately, but two sizes of chromosome. The results, however, do give pencils of sloping lines, and strongly suggest a change in slope unaccompanied by a change in position, i.e. a change in i and not d (Fig. 7 b). These results clearly demonstrate the validity of the foregoing analysis.

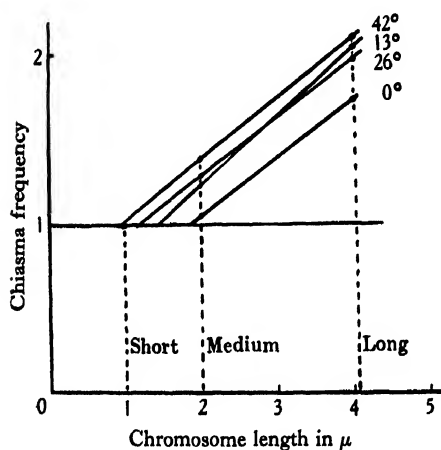


Fig. 7 a

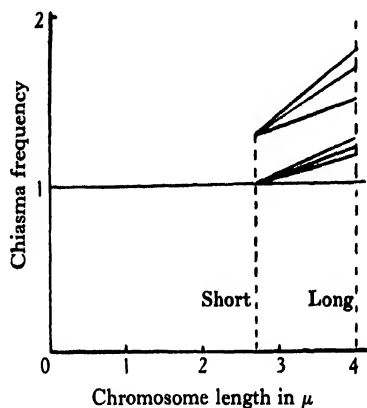


Fig. 7 b

Fig. 7 a. The effect of temperature changes on the chiasma frequency–chromosome length relation in *Locusta*. The change is one of position and not of slope of the lines, i.e. a change in the differential distance only (White, 1934).

Fig. 7 b. The chiasma frequency–chromosome length relation in different individuals of *Culex*. Within each of the two obvious groups the differences are of slope and not of position, so indicating different interference distances with constant differential distance (Moffett, 1936).

An analysis of data on the cytological distribution of crossing-over in *Drosophila melanogaster* confirms and extends the conclusions derived from a consideration of the chromosome length–chiasma frequency curves (Mather, 1936 c). It is clear that some localization must be supposed to occur in this fly in order to explain the differential effects of environmental factors, temperature, X-rays, etc., on crossing-over in various regions of the chromosomes (Plough, 1917; Graubard, 1932; Muller, 1925). The regions near the centromere are affected more than the distal parts. Hence even if the frequency of crossing-over was constant per unit cytological distance along the chromosome under one given set of conditions, it would not be so under others. Therefore localization must occur.

A comparison of the cytological and genetical chromosome maps (Fig. 8) clearly indicates little crossing-over near the centromere and more in the distal regions.

This is equally true whether the chromosome is one or two armed. Thus the centromere must be supposed to play a deterministic function in crossing-over. Confirmation of this is provided by its effects on interference (Graubard, 1934; Mather, 1936 *c*; cf. Schweitzer, 1935), and its effects in a homozygous translocation (Beadle, 1932 *b*). We may consider that crossing-over originates at the centromere and proceeds along the chromosome. This space and time sequence is inferred from the property of interference and from observations on the frequency of crossing-over near the centromere. The validity of the inference is confirmed by an analysis of the distribution of the various chiasmata along the cytological chromosome (Mather, 1936 *c* and unpublished analysis). The full analysis of these data is, however, too heavy to be given in detail here. It is sufficient to say that if there is no regular

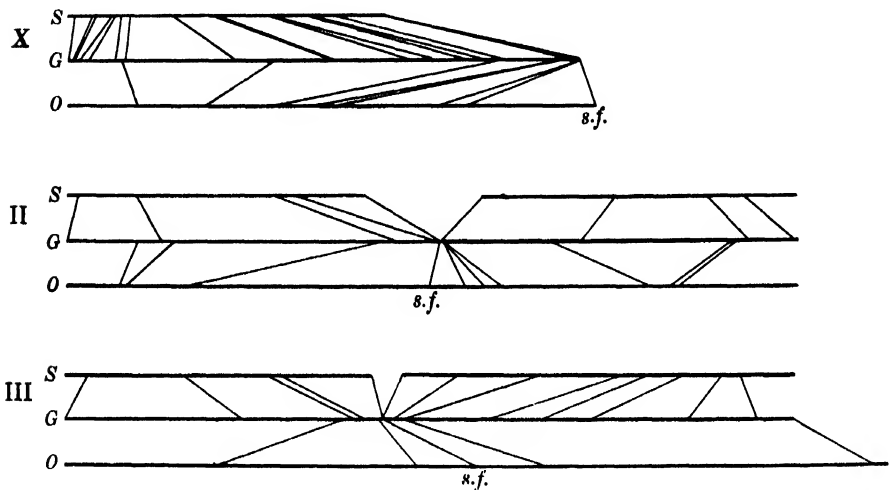


Fig. 8. Genetical (cross-over) and cytological (mitotic and salivary gland chromosome) maps of the three long chromosomes of *Drosophila melanogaster*. The three maps of each chromosome are marked G, O and S respectively. The spindle attachment or centromere is marked s.f. Transverse lines join corresponding loci on the various maps of each chromosome. Data from Dobzhansky (1929, 1930), Muller & Painter (1932), Painter (1934, 1935) and Mackensen (1935) (Mather 1936 *c*).

space and time sequence, when it would in any case be difficult to see how localization could occur, the various chiasmata or points of crossing-over should be distributed along the chromosomes in parallel frequency curves. This is not the case. Some of the curves for distal chiasmata have lower means and greater variances than others, viz. those for proximal chiasmata. Then there must be a time sequence. A comparison of the results of analysis, taking the centromere and the end of the chromosome as origins, shows the centromere to be the real starting-point. Thus one of the corollaries of the hypothesis of position determination is fully confirmed.

We may, by the use of transformation equations based on the binomial $(\frac{1}{2} + \frac{1}{2})^n$ where n is the number of chiasmata (cf. § II (3)), convert multipoint crossing-over data into chiasma frequency data (see also Mather, 1933 *a*, 1935 *c*, 1936 *c*; Weinstein, 1936). In this way separation of the effects of the various chiasmata from one another is possible. Using the centromere as the origin, it is then possible to plot the

frequency of crossing-over due to each individual chiasma, first (proximal), second (next distal), etc., against the cytological distance from the centromere (Mather, 1936 c). By this means sigmoid curves, as shown in Fig. 9 (middle), are obtained.

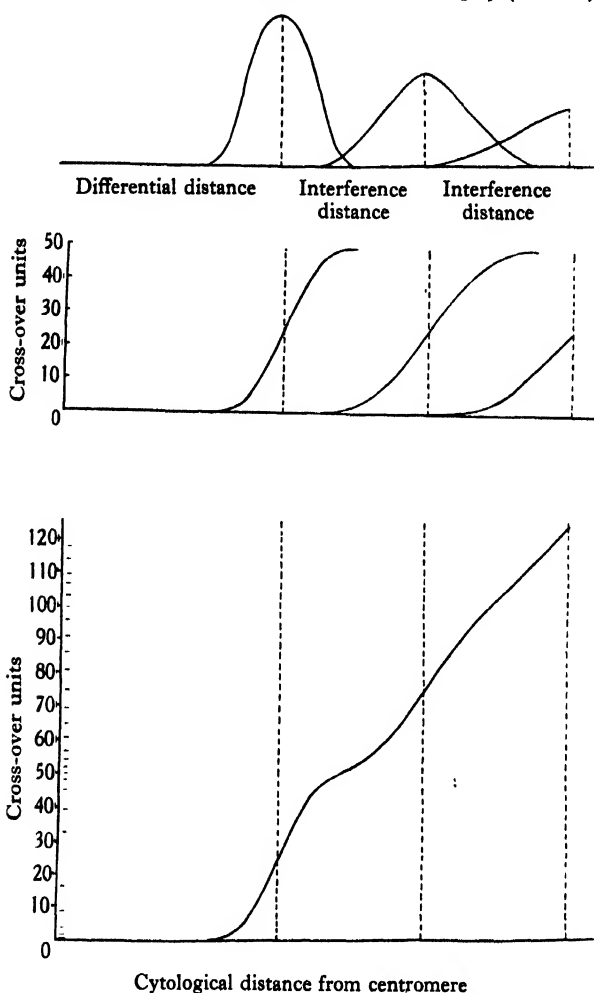


Fig. 9. Diagram to illustrate chiasma localization. Top: the frequency distributions of the first (proximal) and other chiasmata about their mean positions, denoted by dotted lines. The centromere is taken as the origin and the positions of the chiasmata are determined by the differential and interference distances. Middle: the integrals of the above curves to give the relation of genetical (cross-over) to cytological intervals between any points on the chromosome as determined by each chiasma separately. Bottom: the summed integral curves to give the total genetical distance between any two points on the chromosome of any cytological distance apart. The marks up the ordinate are obtained by projecting, via the curve, equally spaced points on the cytological chromosome on to the genetical chromosome. They give an idea of the crowding and spacing effects to be expected in genetical maps as a result of chiasma localization. This agrees well with observation in *Drosophila* (Mather, 1937).

In effect the complex relation of cytological distances and map distance, as shown in Fig. 9 (bottom), has been broken up into its constituent parts. These curves are the integrals of the frequency per unit cytological length curves and the latter can

be obtained by geometric differentiation. They are characteristic unimodal frequency distributions as shown in Fig. 9 (top). Thus the different chiasmata tend to fall in definite places, i.e. are localized, the distribution being determined from the centromere.

A comparison of the mean position of the first chiasmata in different chromosomes shows the differential distance to be variable between the chromosomes, its magnitude being a direct function of chromosome length.

The study of the interference distance is not so easy and its properties cannot be determined so accurately. It does, however, appear to be substantially constant within and between chromosomes (Mather, 1936 *a*; cf. Schweitzer, 1935).

Thus the *Drosophila* data confirm the previous conclusions in all detail, and even go further in relating crossing-over to the centromere.

The cause of the differential reaction of crossing-over in various regions of the chromosomes to environmental changes is now clear. Near the centromere crossing-over per cytological unit is low. If the differential distance is modified slightly by changed environment, the proportionate increase and decrease of crossing-over will be greatest in such a region of low crossing-over. In general the centromeric region should be the most variable in its crossing-over properties, as has, indeed, been shown to be the case by Gowen (1919).

These results relating to localization may be shown diagrammatically as in Fig. 9. The various chiasmata form in frequency distribution with characteristic means, whose cytological positions are governed by the differential and interference distances (Fig. 9 (top)). The second chiasma has a frequency distribution of greater variance than the first because its position is dependent on that of the first one. Its variance will be that of the differential and interference distances jointly. The relation of crossing-over and cytological distance between two points is clearly given by the integral of these curves, because the total crossing-over in any region is obtained by summing the crossing-over in all the unit cytological distances which make up that region. The integral curves are shown in Fig. 9 (middle) for each chiasma separately. When the effects of all the chiasmata are combined by addition, Fig. 9 (bottom), the observable relation is obtained. Assuming that all cytological units of length are equally likely to show mutation, we can obtain an idea of how the genes should show crowding in a genetical map by projecting equally spaced points from the cytological map on to the genetical axis via the curve. The results of doing so are shown in Fig. 9 (bottom) and absolutely reproduce the situation shown by *Drosophila* (see genetical map of *Drosophila* given by Sansome & Philip, 1932).

(5) *The mechanism of crossing-over*

It is now clear that crossing-over happens when the chromosomes split at the end of the pachytene stage of meiotic prophase and that it occurs in certain positions relative to a fixed point which is, in *Drosophila*, at least, the centromere. This knowledge enables us to clarify our position with respect to how crossing-over occurs. Of the various questions that arise in this connexion the following are amongst those of immediate importance:

(a) What is the immediate source of the energy involved in crossing-over, and how is it rendered available?

(b) How does this energy become distributed along the chromosomes, especially with reference to the positions of the chiasmata?

(c) Why does crossing-over always involve two non-identical chromatids at precisely the same level?

No final answer can be given to these questions.

A number of theories of crossing-over have been produced (e.g. Wilson & Morgan, 1920; Belling, 1931, 1933; Darlington, 1935 *a*). That of Wilson & Morgan merely relates crossing-over to torsion in the paired chromosomes. Belling's hypothesis is more of a description of events, and fails to give any account of interference. The mechanism proposed by Darlington is comprehensive but may require some modification, as indeed Darlington (1936 *a*) points out. It does, however, clearly incorporate several principles of the greatest value.

This author relates crossing-over to chromosome coiling. The two homologous chromosomes, as a result of relict coiling in the same direction surviving from the previous mitotic contraction, pair in such a way that their internal and relational coils are in equilibrium. This depends on the assumption of directional specificity in the pairing of the chromosomes which has the result of preventing the paired homologues from slipping round each other. Each chromosome then divides into two thinner and more fragile chromatids and the strain of coiling results in one of the chromatids breaking. This immediately produces a rather complicated readjustment of the forces at that level throwing more strain on to the opposite chromosome and resulting in a non-identical chromatid breaking at this same level. Crossing-over then occurs because the broken ends of one chromatid will revolve in opposite direction and are more likely to encounter ends from the other broken chromatid than to meet one another. Coiling stresses are relieved in this way by crossing-over. The chromosomes show longitudinal cohesion and in consequence reduction of the stress occurs for some distance on either side of the chiasma. This naturally reduces the chance of crossing-over in these regions and results in interference.

Various objections have been raised to this hypothesis by Sax (1936). He claims that evidence for homologous chromosomes always coiling in the same direction is lacking and that, also, Darlington's hypothesis would not account for the observed frequencies of chromatid interlocking at anaphase separation. Such interlocking is another expression of coiling as it occurs when the two chromatid loops, resulting from the formation of two compensating chiasmata, have each one strand passing through the other loop (Fig. 10). This whole question of the direction of coiling in homologues will require further investigation before a final conclusion can be reached (Darlington, 1936 *a*). It does seem clear, on the other hand, that chiasma formation is directly related to coiling, even if the precise details of the relation are not fully known.

It is clear that a large number of the points of Darlington's hypothesis are sound and are of the greatest importance to any reconsideration of the question. His evidence showing how chiasma formation replaces coiling is convincing and supplies

the answer to the question of the immediate origin of the energy necessary for crossing-over. The assumption of the directionally specific action of the chromomere, or whatever the pairing unit is, is in good keeping with general knowledge of chromosome growth and splitting and indicates the means whereby pairing arrests the decay of coiling and so stores up potential energy for crossing-over. His hypothesis that breakage of one chromatid determines, by an upset of equilibrium and resulting transfer of stress, the breakage of a non-identical chromatid at the same level supplies an answer to one of the most troublesome questions of this very obscure subject. Finally the notion of lateral cohesion along the chromosomes supplies a mechanism for interference and is also of further value as will be seen below.

Let us consider the further information relative to crossing-over afforded by the knowledge of position determination. The position of the first chiasma, i.e. its distance from the fixed origin in the chromosome, is a function of the length of the

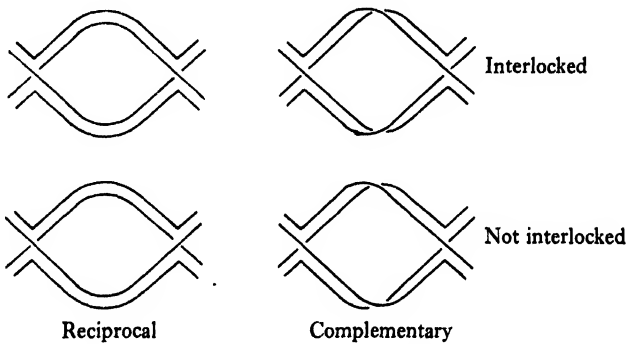


Fig. 10. Chromatid interlocking. The two closed loops produced by a pair of compensating chiasmata are, or are not, interlocked according to whether one of the chromatids of each loop passes through the other loop. This is clearly related to chromatid coiling.

chromosome, but on the other hand the interference distance is either independent of chromosome length or, at least, less highly correlated with it. The process of crossing-over commences at a given origin, i.e. presumably the splitting of the chromosomes into chromatids starts at this point, and runs along the chromosomes (cf. § II (4)). The stress or driving force produced in the chromosomes by a split of unit length must be constant throughout the whole complement as we know that, in *Drosophila*, the differential distance is independent of where the chromatin originated, crossing-over frequency being probably a function of position only. Hence the differences between the chromosomes in the formation of the first chiasma must be a difference in reaction to a given imposed stress rather than to a difference in the stress itself. In other words the chromosomes must be able to take up, or neutralize the effect of part of the imposed stress, by some action of the unsplit portions. The longer the unsplit parts the more of the stress is successfully neutralized and the greater the split portion must grow before a chromatid is broken by the unneutralized stress. Thus the differential distances are determined by the lengths of the chromosomes.

The formation of one chiasma will abolish a portion of the stress but not of necessity the whole of it. The formation of a second chiasma will necessitate the reinforcement of this residuum by further splitting until it, too, reaches the level necessary to cause breakage. As the residuum will on the average be practically constant throughout the chromosomes, the length which must split before a new chiasma can be formed will be considerably less highly correlated with chromosome length than was that necessary for the production of the first chiasma.

Hence there is a difference in behaviour between the differential and interference distances.

This postulated process is supported by various observations. In the first place it supposes that splitting commences at the origin of crossing-over, which has been, in some cases, identified with the centromere (§ II (4)). Cytological evidence of precocious splitting at the centromere has in fact been obtained (Darlington, 1935 *b*; Mather, unpublished).

The assumption that stress can be transmitted along the chromosome is quite justifiable inasmuch as during anaphase separation the chromosomes show themselves capable of withstanding considerable tensions without disruption.

The question of the splitting of the chromosomes releasing the stress which gives rise to crossing-over is more difficult to justify in detail. It is, however, clear from § II (2) that crossing-over occurs at the time of splitting. This marks the end of a stable state and the beginning of the diplotene movements. Hence it is, to say the least, a reasonable assumption.

Finally, there is the question of the accommodation of a portion of the stress by the unsplit parts. Some little evidence in favour of this argument is afforded by the behaviour of triploid *Drosophila*. There is reason to believe that pairing of the chromosomes is incomplete in these, as in other, triploids (Mather, 1933 *a*, 1935 *c*; Beadle, 1934). This is accompanied by increased crossing-over in the regions near the centromere (Redfield, 1930, 1932), i.e. formation of the first chiasma nearer to the centromere, than in the diploid. In other words a change of pairing in the body of the chromosome results in an alteration in the differential distance, so agreeing well with the assumption made. It would then appear that unpaired chromosomes cannot accommodate the stresses to the same extent as paired chromosomes.

These conclusions may be summarized under the headings given earlier in this section.

(*a*) The energy necessary for crossing-over is developed from the coiling of the homologous chromosomes while under the restraint of the directionally specific pairing of pachytene. It is rendered available when splitting of the chromosomes occurs and removes this pairing restraint.

(*b*) This released torsion stress is transmitted along the chromosomes and may be partly accommodated by the remaining paired unsplit portions. The unaccommodated stress accumulates until it is sufficient to cause chromatid breakage. It is then partly released. Further splitting is necessary for the increase of the remaining stress until it is sufficient to cause the formation of a second chiasma. If the chromosome splitting is commencing at some fixed point, e.g. the centromere, and

proceeding regularly along the chromosomes, the positions of the chiasmata will clearly be determined from that point.

(c) Breakage of one chromatid at any level causes, by a readjustment of the forces on the chromosomes, the opposite chromosome to be subject to increased stress. This leads to breakage of a chromatid of this second chromosome at the same level as the first breakage.

Further details of the mechanism of the generation, transmission, and operation of the stress causing crossing-over cannot be obtained without further observation.

One more point requires discussion, viz. the nature of chromatid interference. Again, this cannot be completely described, but some suggestion may be made concerning it. Crossing-over directly concerns but one of the two chromatids from each chromosome. Hence, if they differ in any way, one or other would tend to cross-over with a disproportionately high frequency, and chromatid interference would result. Two obvious possibilities exist. First, that one chromatid is, by its method of formation, thinner and more fragile than the other. This would then tend to break more often and would give an excess of reciprocal crossing-over as is observed in the sex chromosomes of *Drosophila* males. The other possibility is that when one chromatid has broken in crossing-over its internal stress is relieved more than is that of its sister chromatid. Hence it would be less likely to cross-over again and an excess of complementary crossing-over would ensue.

The case of any given bivalent may involve both such possibilities and the final result would then depend on their joint balance.

III. THE EXPRESSION OF CROSSING-OVER

Each of the various expressions of crossing-over shows its own peculiar relation to the underlying common cause. These relations are mathematically very varied, though superficially the effects may be in close resemblance. This has, in the past, sometimes led to erroneous conclusions. Consequently the various forms of expression of crossing-over and their relations to crossing-over itself will be considered in some detail.

(1) *Recombination*

Linkage and crossing-over were first discovered (Bateson and Punnet, cf. Bateson, 1909) by a study of the recombination of genes, and it was by this method that the early work on crossing-over was done.

Mendel showed that independent segregation of factors sometimes occurred, giving the gametic series of **AB** 1, **Ab** 1, **aB** 1, **ab** 1. On the other hand genes may be completely linked in their segregation, giving a gametic ratio of **AB** 1, **Ab** 0, **aB** 0, **ab** 1 or **AB** 0, **Ab** 1, **aB** 1, **ab** 0, as for example the genes yellow and scute in *Drosophila* and *P* (pink) and *B* (bandless) in *Helix hortensis*. The most usual condition with linkage is to find a gametic series of **AB** 1-*p*, **Ab** *p*, **aB** *p*, **ab** 1-*p* where *p* (or 1-*p* in the case of repulsion) is the recombination value and varies between 0 and 0.5. It is very exceptional to find *p* > 0.5.

When the recombination value is small it is equal to the frequency of crossing-over and is then used as a direct measure of crossing-over. Double crossing-over, however, destroys this simple relation when p is larger. The recombination fraction is then less than the frequency of crossing-over by some amount depending on the frequency of double crossing-over which is in turn related to interference.

In the absence of chromatid interference the relation between recombination and crossing-over is simple.

Let the frequency of formation of 0, 1, 2, etc. chiasmata in the chromosome region under consideration be a, b, c, d , etc. Then the frequencies of strands with 0, 1, 2 cross-overs in them will be (from § II (3)):

Chiasmata	Cross-overs			
	0	1	2	3 ...
0	a			
1	$\frac{1}{2}b$	$\frac{1}{2}b$		
2	$\frac{1}{4}c$	$\frac{1}{2}c$	$\frac{1}{4}c$	
3	$\frac{1}{8}d$	$\frac{3}{8}d$	$\frac{3}{8}d$	$\frac{1}{8}d$
⋮				

which on summing gives

0 cross-overs	$a + \frac{1}{2}b + \frac{1}{4}c + \frac{1}{8}d \dots$
1	„ $\frac{1}{2}b + \frac{1}{2}c + \frac{3}{8}d$
2	„ $\frac{1}{4}c + \frac{3}{8}d$
3	„ $\frac{1}{8}d$
⋮	

Now double crossing-over will restore the original relation of the two genes and so will be non-recombination. Similarly triple crossing-over will give recombination, quadruple crossing-over no recombination and so on. Hence the frequency of recombination will be given by the summed frequencies of the single, triple, quintuple, etc. cross-overs, i.e. will be

$$\frac{1}{2}b + \frac{1}{2}c + \frac{1}{2}d \dots$$

Hence as $a + b + c + d \dots = 1$ we may express the recombination value in terms of a alone:

$$p = \frac{\frac{1}{2}b + \frac{1}{2}c + \frac{1}{2}d \dots}{a + b + c + d \dots} \\ = \frac{1}{2}(1 - a),$$

which tends to $\frac{1}{2}$ as a tends to 0 and tends to 0 as a tends to 1.

The chief point to note about this relation is that, in the absence of chromatid interference, the maximum recombination is 0.5 or 50 per cent. This is irrespective of the degree of chiasma interference about which no assumptions were made. This result was arrived at by a different method by Emerson & Rhoades (1933). It is not a proof of four-strand crossing-over as a similar relation is obtainable with certain interference relations where crossing-over is assumed to occur in the two-strand stage (Jennings, 1923; Winge, 1935).

Where chiasma interference is absent, a, b, c, d , etc. are distributed in a Poisson series of form $e^{-m} \left(1, m, \frac{m^2}{2!}, \dots, \frac{m^r}{r!}, \dots \right)$, where m is the mean chiasma frequency.

Then

$$p = \frac{1 - e^{-m}}{2} \\ = \frac{1}{2} (1 - e^{-2x}),$$

where $x = \frac{1}{2}m$ = the mean frequency of crossing-over (Haldane, 1918).

This relation between p , the recombination value, and x , the cross-over value, may be plotted on a graph (Fig. 11). The line $p = x$, i.e. complete interference and no double crossing-over, is also plotted in this figure.

If a given relationship of p and x observed from multipoint experiments is plotted on this same graph it will almost invariably fall between the line $p = x$ and the curve $p = \frac{1}{2} (1 - e^{-2x})$. Exponential curves may be fitted to such empirical data. Methods based on this procedure have been employed by Anderson & Rhoades (1931) and De Winton & Haldane (1935) for the measurement of interference.

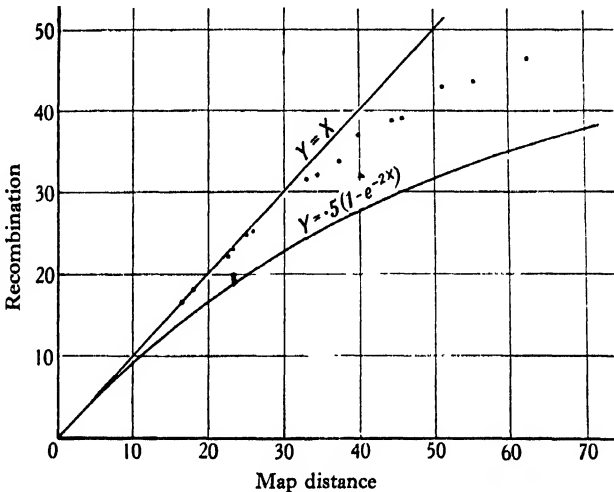


Fig. 11. The relation of recombination to crossing-over (map distance). When there is complete interference the line $y = x$ is obtained and with no interference the curve $y = \frac{1}{2} (1 - e^{-2x})$ holds good. The observed results are shown as dots (Anderson & Rhoades, 1931). Note that the y of this diagram is the p used in the text.

It will be noticed that the above discussion refers only to chiasma interference. Chromatid interference introduces a number of complexities.

In the first place the equation based on the expansion of $(\frac{1}{2} + \frac{1}{2})^n$, relating chiasma frequency and cross-over frequency, no longer holds for $n > 1$. It is not, however, clear what alteration of the equation is necessary. For $n = 2$ the relation could be based on the expansion of $(k + l)^2$, where k is the probability of a strand crossing-over at both or neither of the two chiasmata, and $l (= 1 - k)$ of a strand crossing over at only one chiasma. It is then clear that neglecting all cases of more than two chiasmata, the recombination value will be given by

$$p = \frac{1}{2}b + \frac{1}{2}lc.$$

Clearly the recombination value will depend not only on a as before but also on l . If $a = 0$ and $l > 0.5$ the recombination value will exceed 50 per cent. With $a > 0$

and $l > 0.5$ it may or may not do so. With $l < 0.5$ the upper limit of recombination will be lower than 50 per cent, which is, of course, difficult to distinguish from the case of $a > 0$ and l at its random value of 0.5.

The occurrence of more than 50 per cent recombination is definite evidence of $l > 0.5$ and so of chromatid interference of the type giving an excess of complementary chiasmata. This phenomenon has been observed on a number of occasions though, owing to the slight excess over 50 per cent to be expected from such interference, its significance has not always been beyond question. The factors dilute and wavy in mice have shown this effect (Fisher & Mather, 1936 *a*) but later results reduced the significance of the excess (Fisher & Mather, 1936 *b*). Other cases are those of Wellensiek (1929) in *Pisum* and Clausen (1926) in *Viola*.

(2) Segregation and separation

An expression closely related to recombination in its history and observable results is that of segregation in anomalous cytological types. The mathematical relations are, however, of an essentially different kind.

In the normal diploid organisms each meiotic configuration comprises four chromatids at the time of crossing-over. As four gametes are formed, each germ cell receives one of these chromatids. Hence segregation for a factor, heterozygous in the soma, is always a 1 : 1 ratio irrespective of crossing-over.

In certain aberrant kinds of zygote, however, this situation is not found. One or more of the gametes may receive two or more chromatids from any meiotic configuration. This involves a new variable in the segregation, viz. the type and frequencies of combination of particular portions of the strands to be found in any gamete. It is important to note that segregation will be affected in this way if, and only if, the chromatids under consideration were members of the same configuration of chromosomes at the first meiotic division. If this was not the case, even though the chromosomes be homologous their combination in the gametes will be at random and will not involve consideration of crossing-over.

Examples of such aberrant zygotes are autotetraploids, \widehat{XX} and \widehat{XXY} *Drosophila* and the mosaic strains of *Bombyx mori*.

The occurrence of more than two chromatids in one gamete is dependent on their reaching the same daughter nucleus at the second anaphase of meiosis, which is in turn dependent on them reaching the same interphase nucleus. The whole problem turns on this question of anaphase separation, which may also be studied in certain fungi where the spores result from division occurring in a known linear sequence (Dodge, 1929; Lindegren, 1932).

The relations of the separation of the chromatids to crossing-over is easily seen. Consider the four chromatids composing a bivalent at meiosis. Representing those from one chromosome as **A A** and those from the other as **a a**, there are two possible types of first anaphase separation, the reductional type, **A** and **A** going to one pole and **a** and **a** to the other, and the equational **A** and **a** to each pole. The frequencies of these types of separation at any locus will depend on (*a*) the type of separation at the centromere from which disjunction is controlled, (*b*) the frequency of chias-

mata between the centromere and the locus, (c) the degree of chromatid interference.

Both cytological and genetical data show anaphase separation at the centromere to be entirely reductional, except in the case of univalents. Secondly, chromatid interference may be neglected as it is not known to occur widely, and is very troublesome to incorporate in the analysis. Thus we are left with the consideration of the effects of crossing-over.

If there are two loci **A**, **a** and **B**, **b**, **A** being nearer to the centromere, with one chiasma formed between them, it is easy to see from Fig. 12 (*a*) that where separation is reductional at **A**, **a** it will be equational at **B**, **b**. Where separation is equational at **A**, **a** there are two possibilities. Either the two strands going to the same pole may cross-over at the chiasma between **A** and **B**, when separation will also be equational at **B**, **b**, or crossing-over may occur between strands going to opposite poles, when separation at **B**, **b** will be reductional (Fig. 12 (*b*)). The alternatives will occur equally frequently in the absence of chromatid interference. Hence the amount of reductional separation at a locus is half the equational separation at another locus nearer to the centromere, with one chiasma between them.

Let R_n and E_n be the frequencies of reductional and equational separation at a locus situated so that n chiasmata form between it and the centromere.

$$\begin{aligned} E_n &= 1 - R_n = 1 - \frac{1}{2}E_{n-1} \\ \frac{1}{2}E_n &= \frac{1}{2} - \frac{1}{4}E_{n-1}. \\ \therefore \frac{3}{2}E_n &= 1 + \frac{1}{2} - \frac{3}{4}E_{n-1} \\ &= 1 + \frac{1}{2} - \frac{1}{2}\left(1 + \frac{1}{2} - \frac{3}{4}E_{n-2}\right) \\ &= 1 - \left(\frac{1}{2}\right)^2 - \frac{3}{8}E_{n-2} \\ &= 1 - \left(-\frac{1}{2}\right)^n + \frac{3}{2}\left(-\frac{1}{2}\right)^n E_0 \\ &= 1 - \left(-\frac{1}{2}\right)^n \text{ as } E_0 = 1 - R_0 = 0. \\ \therefore E_n &= \frac{2}{3}\left[1 - \left(-\frac{1}{2}\right)^n\right] = \frac{1 - \left(-\frac{1}{2}\right)^n}{1 - \left(-\frac{1}{2}\right)}. \end{aligned}$$

This is the formula for the sum of the first n terms of the geometric series $\left(-\frac{1}{2}\right)^x$ where x takes the values 1, 2, 3, etc.

This is a very different relation from that of recombination and crossing-over. One immediate consequence is worth noting. In the case of recombination when a , the proportion of bivalents with 0 chiasmata, is 0, $p = 0.5$. In recombination the two genes, marking the ends of the chromosome segment concerned, will then be independent of one another. This is irrespective of the map distance between them, provided, of course, as $a = 0$, that it is at least 50 units.

In the case of separation, however, independence of the behaviour of the centromere, and by analogy of any other locus, is not reached until $\left(-\frac{1}{2}\right)^n = 0$, i.e. $n = \infty$, and is not even closely approximated until $n = 5$ or so. Hence the separations at two gene loci does not approximate to randomness until the intervening distance is high, say 150 or more map units, to allow for the effects of variation. The exception to the rule is when the genes are in different arms of the same chromosome. Their separa-

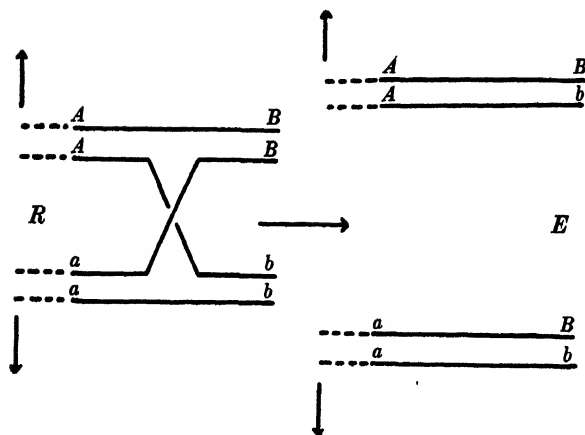


Fig. 12 a

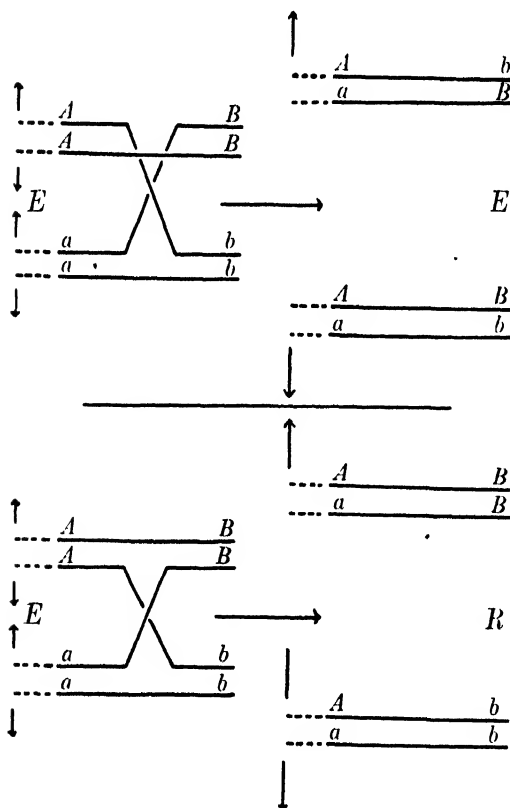


Fig. 12 b

Fig. 12. (a) If the locus *A, a* is separating reductionally and a chiasma forms between *A, a* and *B, b*, then *B, b* will show equational separation. (b) If the locus *A, a* is separating equationally two possibilities are open. Where the strands going to the same pole cross-over again, separation at *B, b* is also equational; but where strands going to opposite poles cross-over the separation at *B, b* will be reductional (Mather, 1935 a).

tion is then at random. Thus the two expressions of crossing-over have very different properties in this respect, a fact that has not been realized until recently. The failure to realize this has led to erroneous conclusions, e.g. the expectation of 16.7 per cent homozygosis at the left end of the chromosome in \widehat{XX} *Drosophila* and the expectation of random chromatid segregation in autopolyploids at distances of 50 or more units from the centromere (see below).

As the amount of equational separation is given by the sum of n terms of a geometrical series of which the constant factor is $(-\frac{1}{2})$ it will oscillate round its limiting value of $\frac{2}{3}$ (reached when n is very large). The natural variation in the frequency of chiasma formation in a chromosome or chromosome arm, will result in the observable oscillation of the degree of equational separation being less violent.

The relation between separation and crossing-over is well demonstrated by the occurrence of homozygosis in \widehat{XX} *Drosophila*. In this line the two X -chromosomes of the female are joined together to a single centromere at their right ("bobbed") ends. They can cross-over just as if not attached, but the four chromatids can separate at but one division. Two of the gametes will each receive a pair of attached X 's and the other two will receive no X -chromosome (L. V. Morgan, 1922; Anderson, 1925). Where the fly is heterozygous for a gene, say **A** and **a**, recessive types will appear in the progeny if **a** allelomorphs are carried by those two chromatids passing into the same gamete, i.e. attached to the same centromere. At the centromere itself non-sister strands are joined. Hence homozygosis or the occurrence of **aa** progeny can only occur if crossing-over has resulted in those portions of the two sister chromatids, from the **a** chromosome, becoming attached to the same centromere. This is clearly a problem in separation and has been worked out along these lines on several occasions (Sax, 1932; Kikkawa, 1933; Mather, 1935 *a*; Beadle & Emerson, 1935; Weinstein, 1936) though seldom with reference to the general problem.

There is one fundamental difference between the separation behaviour of ordinary bivalents and that of attached bivalents. In the former separation at the centromere is reductional, in the latter equational, as two unlike chromatids are attached to the same centromere. Hence a modification of the separation formulae is required. E_0 is now 1 and so, from the previous treatment, E_n is given by the sum of the first $n+1$ terms of the geometrical series.

$$\text{Hence } E_n = \frac{2}{3} (1 - (-\frac{1}{2})^{n+1}) \text{ and } R_n = 1 - \frac{2}{3} [1 - (-\frac{1}{2})^{n+1}].$$

Reductional separation produces homozygosis of one allelomorph or other, but **AA** types are indistinguishable from **Aa** individuals. Hence detectable homozygosis, the occurrence of recessives, **aa**, is half the frequency of reductional separation.

$$\text{Then } H_n = \frac{1}{2} - \frac{1}{3} [1 - (-\frac{1}{2})^{n+1}],$$

where H_n is detectable homozygosis at a locus n chiasmata from the centromere. Table I gives the values of H_n for particular values of n .

Table I

n	0	1	2	3	4	5
H_n	0	$\frac{1}{4}$	$\frac{1}{3}$	$\frac{5}{12}$	$\frac{3}{8}$	$\frac{11}{12}$

It is possible to calculate the chiasma frequency distribution of a chromosome from its cross-over frequency distribution obtained from multipoint cross-over experiments. This has been done for *X*-chromosome of *Drosophila* (data from Morgan *et al.* 1935). The results are given in Table II together with the map distance (amounts of crossing-over) from **bb**, which is genetically coincident with the

Table II. *Chiasma frequency distributions, mean frequencies of crossing-over, recombination and homozygosis for certain regions of the X-chromosome in Drosophila melanogaster. All frequencies are expressed as percentages*

Region	No. of chiasmata per bivalent				Crossing-over	Recombination	Homozygosis
	0	1	2	3			
bb-car	91.19	8.81	—	—	4.41	4.41	2.20
bb-f	76.41	23.46	0.13	—	11.86	11.79	5.88
bb-s	47.17	51.12	1.71	—	27.27	26.41	12.99
bb-v	34.36	60.46	5.13	0.05	35.44	32.82	15.77
bb-ct	20.80	61.63	17.23	0.35	48.56	39.60	17.63
bb-cv	14.68	58.83	25.61	0.80	56.36	42.66	18.07
bb-ec	8.17	53.04	36.37	2.43	66.52	45.92	18.26
bb-sc	5.60	48.49	42.89	3.02	71.67	47.20	18.05

centromere, and the homozygosis expected for each gene. Homozygosis is plotted against map distance in Fig. 13. It will be seen that as far as the curve goes it is of

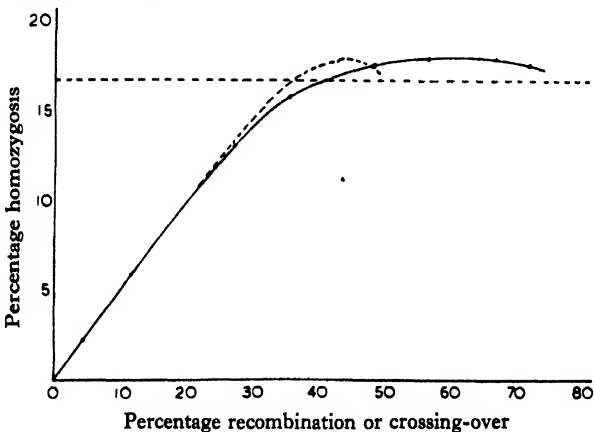


Fig. 13. The relation of homozygosis of genes in the attached *X Drosophila* to crossing-over (solid line) and recombination (broken line) between the gene and the centromere.

the expected type, rising from 0 to a maximum of 18.26 per cent and then dropping. If extended it would presumably oscillate round the limiting value, reached when n is large, of $\frac{1}{2} - \frac{1}{3}$, i.e. $\frac{1}{6}$. That this curve gives a true picture of the situation is shown by Beadle & Emerson (1935) and Mather (1935 *a*).

Fig. 13 also shows homozygosis plotted against recombination of the gene with the centromere. The upper end of the curve is now foreshortened and it is clear that if extended very slightly a point would be reached when recombination is constant but homozygosis variable, so illustrating the different relations of these two expressions with their common cause, crossing-over.

As another example of the importance of the theory of separation in genetics, we may consider segregation in autotetraploids. Each meiotic configuration has eight chromatids of which each gamete receives two. Let us take the general case of each homologous chromosome being marked by a distinct allelomorph $a_1 a_2 a_3 a_4$ at the locus in question. Where the two chromatids of a chromosome never reach the same gamete (locus a in Fig. 14) a gametic series of $a_1 a_2$, $a_1 a_3$, $a_1 a_4$, $a_2 a_3$, $a_2 a_4$, $a_3 a_4$ in equal numbers is obtained. But if two chromatids from one chromosome and reach the same gamete then types $a_1 a_1$, $a_2 a_2$, $a_3 a_3$, $a_4 a_4$ may occur.

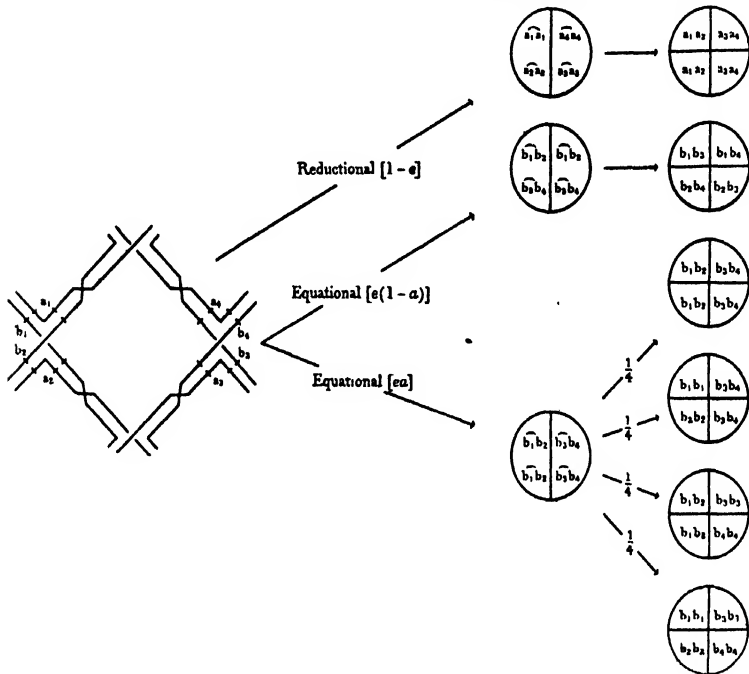


Fig. 14. Segregation in autotetraploids. Where no chiasma forms between the locus (a) and the centromere separation is reductional and the gametic series consists of $a_1 a_2$, $a_1 a_3$, $a_1 a_4$, $a_2 a_3$, $a_2 a_4$, $a_3 a_4$, in equal numbers. Where a chiasma does form between the locus (b) and the centromere the series is still as above if disjunction occurs, but will include gametes $b_1 b_1$, $b_2 b_2$, $b_3 b_3$, $b_4 b_4$ if non-disjunction takes place. The allelomorphs joined to the same centromere at interphase are shown linked and these must pass into opposite gametes at second anaphase (Mather, 1936 *b*).

The frequency of occurrence of these exceptional types will depend on the two chromatids reaching the same interphase nucleus but on different centromeres, as the two chromatids of each centromere pass to opposite nuclei at second anaphase. This will clearly demand (i) equational separation at the locus under consideration, and (ii) non-disjunction of the two chromosomes showing equational separation (locus b in Fig. 14). Hence the exceptional type will occur with a frequency dependent on ea where e is the mean equational separation at the locus and a the mean non-disjunction of adjacent chromosomes. The factor e is variable along the chromosome as shown above and also with environment as it is dependent on crossing-over. Thus there is no simple expectation for the segregation of a gene in a tetraploid

(Mather, 1936 *b*). Variant gene ratios in tetraploids have been recorded in *Rubus* (Crane & Darlington, 1932) and *Solanum* (Lindstrom, 1932; Sansome, 1933). The limiting segregation when disjunction and separation are both at random depends on $ea = \frac{2}{3}$ but this is probably never reached in practice. The observed values of ea may approximate to $\frac{2}{3}$ though not because of random separation and disjunction, but because theoretically a may vary from 0 to 0.5 and e from 0 to 1.

Upcott (1937) has shown that the frequency of production of dicentric chromosome bridges at first and second division of meiosis is directly related to the type of separation. It may also be noted that homozygosis following equational separation may lead to segregation in apomictic individuals producing diploid gametes by the suppression of first anaphase (Darlington, 1936 *c*; Gustafsson, 1934; Bergman, 1934).

Just as in the case of recombination, departures from the above mathematical relations of crossing-over and separation are often evidence of chromatid interference. Such evidence has perhaps been obtained in *Drosophila* (Bonnier & Nordenskiöld, 1937).

(3) *Chromosome pairing*

It is a commonplace of cytological observation that, in the great majority of organisms, chromosomes are paired at the first meiotic metaphase by virtue of the chiasmata formed between them. In other words, crossing-over is essential to metaphase pairing and its concomitant, anaphase disjunction.

Cytologically the situation is clear. If chiasmata are formed the chromosomes, at least in a diploid, will always disjoin at anaphase. In a polyploid with multivalents this statement must be modified to "will also be potentially capable of disjoining". With failure of chiasma formation univalents are formed and, being independent of one another in movement, may reach the same gamete unless an alternative method of pairing is possible as in *Drosophila* males. The cause of failure of chiasma formation is irrelevant. It may be due to genuine failure of chiasmata to form in paired chromosomes, as occurs rarely in normal *Secale* (Mather & Lamm, 1935; Lamm, 1936), but this will almost certainly be uncommon (Mather, 1937). It may be due to failure of pachytene pairing as is the case in triploid species hybrids in *Triticum* (Mather, 1935 *b*) or in plants with reduplications in fragment form (Darlington, 1930 *a*; Mather, 1935 *d*; cf. Mather, 1937). However, the general rule is that chiasma formation is essential to the regular behaviour of chromosomes in meiotic pairing and disjunction and so is essential to fertility, unless the irregularly behaving chromosomes are inert, as are, for example, the *B* chromosomes of maize (Randolph, 1928). The relation between crossing-over and pairing is thus very simple. The frequency with which chromosomes pair is proportional to the frequency of formation of the first chiasma. The pairing is invariable when the chiasma frequency is greater than one, irrespective of the variation of the latter.

Genetically the situation is not so easy to analyse. Crossing-over is judged from the recombination or segregation of genes. If lethal, cross-over strands may not be recovered and so crossing-over will be apparently zero. This has led to the argument

that, as heterozygous inversions "prevent" crossing-over without increasing non-disjunction, chiasma formation is not the necessary and sufficient condition of pairing (Glass, 1935). It has, however, been shown by Grüneberg (1935), Stone & Thomas (1935), Beadle & Sturtevant (1935) and Sturtevant & Beadle (1936) that apparent failure of crossing-over in inversion heterozygotes is due solely to the failure to recover single-cross-over strands. Hence crossing-over does occur, although Sturtevant & Beadle (1936) have concluded that it still fails to account for all the disjunction observed.

The general relation between crossing-over and disjunction is exemplified in *Drosophila* by

(i) The increase of non-disjunction when selection is made for low crossing-over (Detlefsen & Roberts, 1921).

(ii) The correlated decrease of crossing-over and increase of non-disjunction in a special line with genetically changed *X*-chromosome (Anderson, 1929).

(iii) The close relation between crossing-over and disjunction in an interchange heterozygote (Dobzhansky, 1933).

The last example was so striking that Dobzhansky wrote: "Chromosomes which undergo crossing-over usually disjoin normally and those which do not undergo crossing-over are likely to pass to the same pole at reduction division." Thus there can be little doubt of the importance of crossing-over for pairing in the female *Drosophila*. The situation is precisely that observed cytologically in numerous other organisms.

The male *Drosophila* is an anomalous type and does not behave in the same way, so leading to an interesting comparison with the female. It shows practically no crossing-over. This is due to the failure of chiasma formation and the occurrence pairing by affinity in the autosomes, while the *X*- and *Y*-chromosomes pair by two reciprocal chiasmata (Darlington, 1934; Dobzhansky, 1934). The crossing-over resulting from these chiasmata in the sex chromosomes is occasionally detectable (Philip, 1935).

In view of this difference between the sexes in crossing-over a difference in chromosome pairing is to be expected. This is found to be the case. The male is always found to show the same irregularities of pairing and disjunction as a similar female, but also always to a considerably lower degree. For example, in the interchange line considered above, Dobzhansky found the male to give departures from random segregation of the four chromosomes of the ring in the same direction as, but to a considerably less extent than, the female.

Such differences are to be expected if the pairing by affinity in the male is similar to pachytene pairing in the female. The chiasmata form only between associated parts and they will serve to magnify the differences in pairing because chiasmata will very rarely form in sections containing but little association.

An interesting sidelight is thrown on pairing in the small fourth chromosome. Sturtevant (1934, 1936) finds that, in triplo-IV flies there is preferential segregation. This occurs in both sexes but, as usual, the departure from randomness is smaller in the male. Segregation is conditioned by metaphase pairing and so we may infer

a difference in the manner of pairing of the IVth chromosomes in the two sexes. By analogy with Dobzhansky's case the IVth chromosome may then be expected to pair by chiasmata in the female but not in the male. The demonstration of localized chiasmata in the female *Drosophila* (§ II (4)) can reconcile this with the almost complete failure of recombination in the IV chromosomes. It would be interesting to know if non-disjunction of the IV chromosomes is higher in the normal female than in the normal male, as should be the case if this hypothesis of the pairing difference is true.

Finally we have the case of pairing in polyploids. In multivalents the cytologically observable manner of segregation is clearly dependent on the numbers and position of the chiasmata (Darlington, 1934). Two chromosomes are more likely to pass to opposite poles if they take part in a chiasma near to their centromeres. The effects of this are to be seen in Redfield's (1930, 1932) data on crossing-over in triploid *Drosophila*. The gametes carrying two of the three chromosomes show, when averaged, less crossing-over near the centromere than those carrying but one chromosome (Rhoades, 1933). This is because the two chromosomes crossing-over near the centromere pass to opposite poles when, of course, the third must go with one of them. The effect of the third chromosome is to lower the average detectable crossing-over in the gamete carrying it. The extreme case of this behaviour is found when the trivalent is replaced by a bivalent and univalent (Mather, 1933 a).

A somewhat different case is the relation between crossing-over and crowding in the metaphase plate described by Beadle (1935). Its significance is not completely clear and the whole question is in need of further observation.

(4) *Structural change*

So far crossing-over between completely homologous chromosomes has been considered. It may also occur between small segments, homologous but dislocated with respect to each other.

Heritable changes have been classified into two groups: (i) intragenic changes or point mutations, and (ii) alterations in the linear order of the genetical material of the chromosomes, or structural changes. This is a convenient classification, although recent work (Muller *et al.* 1935) has rendered it doubtful whether point mutation can be considered as entirely distinct from minute structural changes.

The major structural changes may be grouped according to whether they are primary, i.e. originating by a chance combination of circumstances, or secondary, derived from primaries by crossing-over.

The mechanism of primary structural change is still not clear. Various hypotheses have been put forward to account for them, particularly with reference to the manner of breakage and rejoining of the chromosome and the differential behaviour of various sections of a chromosome (Stadler, 1931; Catcheside, 1935; Husted, 1936; cf. Darlington, 1936 c). This problem is clearly related to some extent to that of the mechanism of crossing-over, although how nearly is an unanswered question.

The relation of secondary structural change to crossing-over is more precisely

known. Certain structurally heterozygous types throw "mutants" with a low but constant frequency. The classical example of this is provided by *Oenothera*. The mutants, half mutants and mass mutants are all known to be direct or indirect products of crossing-over between non-adjacent chromosomes of the ring (Darlington, 1929 *b*, 1931 *b*). In many cases the occurrence of a chiasma between non-adjacent chromosomes will break down the balanced lethal system and allow of the formation of homozygous types, e.g. the full mutant *deserens* derived by selfing half-mutant *rubrinervis* of *Lamarkiana*. Numerous such examples of secondary structural change, accompanied by physiological change, could be cited. In these cases the production of secondary structural types is really the recombination of whole segments of unrelated chromosome and so will be related to the frequency of crossing-over exactly as is recombination.

It may be argued, however, that *Oenothera* is unusual in this respect of giving numerous secondary types by crossing-over. It is possibly unusual in giving such a high frequency of them, but their occurrence has been demonstrated in at least one other organism. The production of secondary types by crossing-over between non-adjacent chromosomes of a ring has been observed in *Pisum* (E. R. Sansome, 1932, 1933). It may be inferred that it is common to all multiple ring-forming organisms.

Another type of structural change, commonly met with, viz. inversion of chromosome segments, is also possibly fairly frequently secondary in origin. Grüneberg (1935) described a long inversion, accompanied by a change in the eye surface, in the *X*-chromosome of *Drosophila*. More recently (1936) he claims a reversion of the eye surface to wild type, accompanied by reinversion of the chromosome. If correct it seems likely that this inversion became restored to its former condition, and consequently also probably arose, as a result of secondary change by crossing-over. The chance of accurate reinversion of a spontaneous and fortuitous character is too remote to consider.

The case of double-Bar in *Drosophila* is of interest in this respect. Double-Bar arises, by unequal crossing-over, from single-Bar. It can also revert by crossing-over (Sturtevant, 1925, 1928). Double-Bar is a duplication of Bar in one chromosome (cf. Dobzhansky, 1936).

Similar examples are being encountered fairly frequently.

The regular occurrence of such secondary structural changes in apparently normal diploid individuals argues the presence of unsuspected aberrant linear arrangements, possibly of a very small magnitude. Darlington (1932) had indeed predicted the finding of small duplications on general grounds. Evidence has now begun to accrue of their presence in *Drosophila*. Studies of the salivary gland chromosomes with their detailed band structure have provided evidence for the presence of duplications (Bridges *et al.* 1936). Muller (1935 *b*) and Demerec & Hoover (1936) have also obtained similar evidence from the occurrence of flies homozygous for small deficiencies. Furthermore it seems likely that some such duplicated segments, occurring in the same chromosome, are sometimes inverted with respect to one another, in the manner that would allow of inversion and reinversion as found by Grüneberg (1935).

Purely cytological evidence of duplication is afforded by pairing within the haploid set of chromosomes (Catcheside, 1932). Pairing between such segments is commonly found only in haploids and, possibly, triploids because the action of differential affinity renders it extremely rare in diploids, where completely homologous partners are present. Secondary structural change must, however, occur regularly in many organisms.

A commonly occurring type of structural change is inversion (Darlington, 1936 c; Richardson, 1936; Upcott, 1937, etc.). Crossing-over in an inversion leads to the formation of a chromatid with two centromeres. These pass to opposite poles at either first or second anaphase of meiosis and the chromatid may break under the strain, so producing new types of chromosome. This may be a common method of structural rearrangement in the wild. In nature it is partly secondary, in arising by crossing-over, and partly primary, depending on breakage.

Structural changes may be physiologically effective in two ways, either by involving loss or increase of certain regions, or by an accompanying position effect (cf. Dobzhansky, 1936). It seems likely that all or nearly all structural changes do have physiological effects whether in the homo- or heterozygous conditions. Hence secondary, and of course primary, too, structural changes are a source of variation and the materials for the operation of natural selection. Inasmuch as crossing-over is the mechanism of secondary structural change it has the effect of not only producing new combinations of existing types but also of producing entirely new forms. Although secondary structural change is relatively rare, the production of new balances and fresh physiological complexes, which accompany it, is an important source of variation that cannot be overlooked.

IV. THE SELECTIVE CONTROL OF CROSSING-OVER

As crossing-over occurs at an early stage of the first meiotic division its effects may be observed in the gametes of the individual concerned or in the somata and gametogeneses of individuals of later generations.

Of the four types of expression considered above that of pairing affects the gametes of the individual showing the crossing-over. Failure of chiasma formation leads to failure of pairing, which, in turn, leads to failure of regular disjunction and to gametic unbalance. This is a source of sterility, both gametic and zygotic, and in the case of some hybrids the sterility from this cause may be complete. However, other methods of pairing may and do exist, as in the *Drosophila* male and possibly Lepidopteran females. In these cases crossing-over as a method of chromosome pairing is found in the other sex. Consequently it is clear that the essential function of crossing-over is not to promote pairing, as even in species capable of pairing, and pairing more regularly (§ III (3)), by other means, crossing-over is still maintained in some individuals. Hence the survival and frequency of crossing-over will not be governed entirely or even largely by its effect in controlling pairing. If pairing is by chiasmata it is notable, however, that a minimum of 1 per bivalent is essential to full fertility. The need for regular pairing would not appear to have any result other than to favour this minimum.

The effect of crossing-over in causing recombination in the next generation is clearly of fundamental importance. Recombination can regularly occur in no other way. There must be some advantage in regular recombination as it will prevent clonal reproduction of the chromosomes, which would entirely obstruct the combination of advantageous mutants. The necessity for recombination in this way was noted by Weissman as long ago as 1892. It is, however, equally clear, as Fisher (1930) pointed out, that recombination has also a disadvantageous effect in causing breaking up of selectively advantageous combinations of genes. These two opposite tendencies may be expected to reach a selective balance in any organism. A full discussion of this involved question is not possible here but reference may be made to Fisher (1930) and Sturtevant and Mather (1938). In this connexion it is of interest to note that the majority of organisms show chiasma frequencies per bivalent of about 2. Some show much higher frequencies and a few, with short chromosomes, show lower. The general impression given by a consideration of all the data available is, however, that the frequency of chiasma formation per bivalent tends to be about the same no matter what the number of bivalents formed may be. This is, of course, subject to the action of competition which will modify the situation somewhat (Mather, 1936*a*). However, it may be safely said that any balance of the two tendencies discussed above, leading to an optimum chiasma frequency, is a balance within the bivalent and largely independent of the number of bivalents.

Exceptional segregation, as an expression of crossing-over, is apparently a phenomenon of less wide-spread importance, because it can only occur in types with anomalous cytological constitution and behaviour. It certainly allows of segregation in apomictic species, or strains of a species, when these show suppression of the first anaphase leading to the formation of diploid eggs. Its importance in this respect has been noted by Darlington (1936*c*) and Gustafsson (1934). It may be generally inferred that this expression of crossing-over would tend to be of increasing selective advantage as the crossing-over near the centromere increased in frequency, in those cases where it is important.

Secondary structural change, as a result of crossing-over, depends on the pairing at pachytene of, usually, small dislocated homologous segments. The frequency of chiasma formation will not be such an important factor in this process as the frequency of pachytene pairing. It is for this reason that secondary structural change occurs much more freely in a haploid with low chiasma formation than in a diploid with high frequency of chiasmata. Hence this phenomenon will have but little effect in determining the frequency of crossing-over.

Thus pairing, though usually, is not essentially by chiasmata, and in any case demands but 1 chiasma per bivalent; segregation and variation by crossing-over appear to be either of but limited occurrence or largely dependent on other controlling factors. It would then appear that the frequency of crossing-over will be largely governed by its effect in recombination. The measurement and analysis of this property have yet to be achieved, but it appears that it will be a fundamental problem of inheritance in evolution.

V. SUMMARY

The study of crossing-over leads us on the one hand to consideration of the physio-chemical nature and behaviour of the chromosomes, and, on the other to conclusions respecting the better-known phenomena of segregation, pairing, recombination and gene action. Although many aspects of the cause and consequence of crossing-over are still not fully analysed, important conclusions have already been reached.

The chiasmatype theory, that crossing-over is the sole condition of chiasma formation, allows of the accurate co-ordination of the genetical and cytological methods of study of crossing-over. The genetical method consists of marking alternative chromosomes by the use of gene allelomorphs, the segregation and recombination of which may be followed. The cytological method is that of direct observation. The two methods are complementary in their natures.

Crossing-over occurs when the pachytene chromosomes divide and tends to occur in definite positions in the chromosome, as a result of a special time and spacial sequence in formation. The positions are related to fixed points, viz. the centromeres and, possibly, the ends of the chromosomes.

The movements of the chromosomes in crossing-over are related to coiling although the generation and distribution of the energy are not fully understood. Pachytene pairing is directionally specific, i.e. the chromomeres or pairing units are asymmetrical in type. The chromosomes show definite longitudinal cohesion and can transmit stresses along their length. Splitting of the chromosomes generates or releases such stresses, which may be transmitted and partially accommodated in unsplit regions. The unaccommodated stresses result in crossing-over.

The frequency of recombination of loci is related algebraically to the frequency of crossing-over between them by the alternate terms of a binomial expansion. The relation may be expressed empirically in a logarithmic form. In the absence of chromatid interference recombination cannot exceed 50 per cent. Chromatid interference may result in a slight increase of this level.

The frequency of reductional and equational separation of a locus depends on the behaviour at the centromere and on the frequency of crossing-over between the locus and the centromere. The relation is of the form of a summed geometrical series. Segregation in aberrant diploid and in autopolyploid types depends on the frequency of these types of separation. Although the limiting value of recombination may be reached when the loci concerned are but little more than 50 map units apart, they may still show correlated separation.

Metaphase pairing and anaphase disjunction of the meiotic chromosomes is dependent, in the vast majority of cases, on chiasma formation. The regular occurrence of crossing-over is almost universally necessary for full fertility. In cases where the sexes differ in the manner of meiotic pairing, notably *Drosophila*, there is a corresponding variation in segregation, recombination, and disjunction.

The presumably widespread occurrence of primary structural change, mainly duplication and inversion, leads to the regular occurrence of variant types by

crossing-over. These, as in *Oenothera* and *Drosophila*, often have distinctive physiological characteristics and so are a source of material for the action of natural selection.

The frequency of crossing-over per bivalent is open to selective action, and probably reaches an optimum value in any organism. This will chiefly result from its effects on the recombination of advantageous mutants.

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HOMOLOGOUS AND ANALOGOUS MORPHOLOGICAL MUTATIONS IN RODENTS

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I. INTRODUCTION

OF the mammals used in biological laboratories the four rodents which have been studied most extensively are the house mouse (*Mus musculus*), the rat (*Rattus norvegicus*), the guinea-pig (*Cavia porcellus*) and the rabbit (*Oryctolagus cuniculus*). In addition, deer mice of the genus *Peromyscus* have been investigated considerably, although to an extent scarcely comparable with the species named above.

The house mouse and the rat are taxonomically, and presumably phylogenetically, rather closely related, for both are members of the family Muridae, comprising the Old World rats and mice. The guinea-pig is but a distant relative, belonging to the widely separated family Caviidae, while the rabbit probably occupies a still more remote branch of the phylogenetic tree since, strictly speaking, it is not a rodent but a member of the order Lagomorpha. In general usage, nevertheless, the rabbit is termed a rodent. Because of their extensive use as laboratory animals, it is not surprising that more mutations have been recognized and studied genetically in the rat, mouse, guinea-pig and rabbit than in other members of their orders.

Haldane (1927) listed the mutations affecting colour reported in the four rodents

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cited, as well as colour genes in other rodents and carnivores, noting a number of probable gene homologies. He laid down several criteria suggestive of homology, among which were: (a) the production of similar somatic effects, (b) the uniqueness within each of the species compared of a gene bringing about a certain result, (c) more or less parallel series of multiple alleles, and (d) similar linkage relationships. Gene homologies in allied species consequently afford evidence of a common ancestry, for such species presumably possess homologous normal genes, the changes from the ancestral protogenes having resulted from mutations, either singly or in series, during the course of evolutionary history.

Besides the genes affecting colour, which Haldane considered, many morphological mutations have appeared in laboratory rodents. Homology in such mutations gives perhaps even more definite evidence for common protogenes than homology of colour mutations, for the latter are usually more superficial, occurring late in the development of the organism with a resulting shorter chain of reactions between primary gene action and the final observed effect. Consequently, morphological mutations in which the genes responsible act at comparable stages in development, are inherited alike (showing similar dominance and linkage relations) and have a similar somatic appearance, can probably in most cases be produced only if the normal genes in the allied species are homologous or alike. Mutations fulfilling these requirements can be considered homologous as opposed to analogous mutations which present a similar final effect but are produced through quite different means. Morphological mutations of both types are encountered among rodents.

Of the criteria postulated, that of like linkage is perhaps the least dependable since with different chromosome numbers in the four rodents (mouse, 20; rat, 21; guinea-pig, 30-32; rabbit, 22) it is evident that changes have occurred in the ancestral haploid number through fragmentation, combination, or other means, with a consequent altering of original linkage relationships.

As Haldane has pointed out, there is no absolute criterion of homology, and, moreover, identity of genes producing identical effects even in the same species cannot be demonstrated. Nevertheless, certain mutations in related species have such similar characteristics during ontogeny that the final resemblances are explicable by appealing to the principle of homology. On the contrary, other mutations, although similar in their final observed effects, reach the end results by such different paths that the principle of homology is clearly inapplicable; these, as stated above, may be termed analogous.

Morphological mutations affecting nearly all parts of the body have been reported in mammals. Since the development of the organism is so sensitively organized in relation to time and space, any mutation upsetting this balanced rhythm is likely to prove lethal to the embryo if it changes the fundamental plan. This, together with the obviously greater ease of recognizing external changes, may explain the circumstance that most viable structural mutations are relatively trivial and affect external organs.

In the following discussion of morphological mutations, special attention will be given those found in rodents, although comparisons will be made with more or

less similar structural changes which have occurred among other mammals. More detailed descriptions, however, will be reserved for mutations occurring among the four laboratory rodents. For these, moreover, the criteria of homology will be considered and the modes of inheritance discussed as far as known. The account of mutations, as shown in Table I, will be given according to the anatomical regions chiefly affected, beginning with "dwarf" mutants, in which the observed effect is general instead of local.

Table I. *Morphological mutations in rodents*

Region	Mutation	Mouse	Rat	Guinea-pig	Rabbit	Other mammals
General	Dwarf	<i>r</i>	<i>r</i>	<i>r</i>	<i>D</i>	—
	Grey-lethal	<i>r</i>	—	—	—	—
Head	Otocephaly	<i>x</i>	—	<i>x</i>	—	—
	Hydrocephaly	<i>r, r</i>	<i>x</i>	—	—	—
	Hare-lip, cleft palate	<i>r</i>	—	—	—	Swine
	Blindness, microphthalmia, etc.	<i>r, x, x, x, x, x</i>	<i>x</i>	<i>x, x</i>	<i>x, x</i>	—
	Rodless retina	<i>r</i>	—	—	—	—
	Short ears	<i>r</i>	—	—	—	Sheep
	Defective ears	<i>x, x</i>	—	—	—	Swine
	Absence of corpus callosum	<i>r</i>	—	—	—	—
Pelage	Hypotrichosis	<i>D, r, r</i>	<i>r</i>	—	<i>r</i>	Peromyscus, swine, sheep, cattle
	Angora	—	—	<i>r</i>	<i>r</i>	Cat
	Rex, "rexoid"	<i>r, r</i>	<i>D, D, r</i>	—	<i>r, r, r</i>	Woodchuck, swine
	Rough	—	—	<i>D</i>	—	Swine
Tail	Brachyury	<i>D, r</i>	<i>x</i>	—	—	Cat, dog
	Flexed, kinky, etc.	<i>D, r</i>	—	—	—	Swine, cattle
Feet	Polydactyly	<i>x</i>	—	<i>D, x, x</i>	—	Cat, cattle, swine
	Syndactyly, defective feet, etc.	<i>r</i>	—	—	<i>r</i>	Swine, cat
Mammae	Supernumerary	—	—	<i>x</i>	—	Swine, sheep, woodchuck
Viscera	Notched spleen	<i>x</i>	—	—	—	—
	Absence of kidney	<i>x</i>	—	—	—	—
Behaviour	Waltzing	<i>r</i>	<i>r</i>	—	<i>x</i>	—
	Shaker	<i>r, r</i>	—	—	—	—
	Palsy, epilepsy	<i>x</i>	—	<i>r</i>	—	Goat

D = Mendelian dominant; *r* = Mendelian recessive; *x* = Irregular in inheritance.

II. GENERAL MUTATIONS

Dwarf animals are much smaller than the normal individuals of their race, with the difference in size conditioned by a single gene mutation with simple Mendelian inheritance. This situation holds generally in the four rodents under consideration. On the other hand, pigmy races, such as are found among rabbits, differ from large races in numerous genes influencing growth with resulting blending inheritance. The small *Mus bactrianus* similarly differs thus from its larger congener, *Mus musculus*, while a like situation prevails in the case of the guinea-pig and the wild form, *Cavia rufescens*.

In the dwarf mouse, reported by Snell (1929), growth practically ceases at the end of the second week, leaving the animal in an infantile condition. The dwarf gene is completely recessive to its normal allele and when present in a homozygous condition produces both small size and sterility. The marked reduction in growth, as Smith & MacDowell (1930) have determined, results from an anterior pituitary deficiency which can be remedied by daily implants of fresh rat anterior lobe. Such treated dwarfs then resume growth and overcome their sterility.

The behaviour of a dwarf gene in the rat, discovered by Lambert & Sciuchetti (1935), simulates that of the mouse in most respects. The final size attained, however, is relatively somewhat greater, for the affected animals continue to grow slowly after the onset of the retardation. Moreover, preliminary implants of normal rat pituitary bodies failed to induce accelerated growth or fertility. As in the mouse, the dwarf complex behaves as a simple autosomal recessive.

The dwarf guinea-pig, described by Sollas (1909, 1914), suggests a form of achondroplasia, thus differing from the two examples described above. Like them, nevertheless, it behaves in inheritance as a Mendelian recessive.

Dwarf in the rabbit, reported by Greene *et al.* (1934), differs markedly from that in the mouse, rat or guinea-pig, since the mutant gene exerts a dwarfing effect on the heterozygotes, not however rendering them sterile, but kills the homozygotes. It thus belongs to the class of mutations termed dominant lethals.

With the possible exception of dwarfing in mice and rats, none of the four mutations producing like effects can be considered homologous. Even in the two murids, further evidence is needed to establish homology of their dwarf mutations. It may be that dwarf in the rat is a higher allele of that found in the mouse. Too, the discrepancy in the reaction of the two forms to pituitary implants might be obviated if gland tissue from some mammal other than the rat were implanted into dwarfs of that species since the foreign tissue would then be rapidly resorbed with no tendency to grow as might happen if the tissue came from an individual closely related to the host.

A second gene in the mouse bringing about a cessation of growth is the autosomal recessive, grey-lethal, described by Grüneberg (1935), which produces, in addition to its effect on coat colour, loss of weight after the age of 2 or 3 weeks with marked retrogressive effects on skeleton and teeth, the morphological anomalies of which result from the complete absence of secondary bone absorption. This loss of weight culminates in the death of the homozygote usually between the ages of 22 and 30 days.

As an antithesis to dwarfing, mice with the dominant lethal gene for yellow coat colour (A^Y) tend to become extremely adipose with advancing age. In no other rodent is a comparable condition found for only the mouse possesses that agouti allele.

III. MUTATIONS OF THE HEAD

Wright (1934) gave an account of the morphology and heredity of otocephaly in the guinea-pig. This abnormality varies greatly in expression; the lowest grade manifests merely a reduced lower jaw while the highest grade comprises nearly

complete acephaly. Otocephaly is complex in its inheritance, for non-genetic factors play a greater part than genetic although the incidence is markedly influenced by at least one dominant gene.

Among other rodents only in the mouse has a mutation resembling otocephaly arisen. Little & Bagg (1924) described a lethal head abnormality which they believed behaved as a Mendelian recessive. Otocephaly of a low grade (agnathia) occurs sporadically, however, in several other stocks of mice—one example having been found in *Mus bactrianus*—in which its manner of inheritance appears less simple. Since the genetic basis of this mutation is ordinarily so involved, there is little evidence that the basic gene changes are homologous.

Hydrocephaly has been reported twice in the mouse (Clark, 1935; Zimmerman, 1933) and once in the rat (Colton, 1929). In the former murid, the two mutations were phenotypically similar, although the affected animals had different early growth rates, and both were inherited as simple recessives, but independently. A hydrocephalous rat seems to have appeared but once with the condition either not hereditary or due to more than a single gene difference.

Reed & Snell (1931) noted that hare-lip in the mouse was conditioned by a recessive gene subject to a high percentage of normal overlaps. The new-born mice manifesting this abnormality rarely survived more than 24 hours because of inability to nurse. In no other rodent has hare-lip or cleft palate been reported, although Koch & Neumüller (1932) described examples in swine in which the malformation was hereditary.

Blindness, microphthalmia, anophthalmia and other eye defects have appeared in all four laboratory rodents. Little & Bagg (1924) gave an account of an eye defect in the mouse which was a simple Mendelian recessive with, however, the penetrance¹ less than 100 per cent. Little (unpublished) has found a type of blindness which seems to be due to two recessive genes acting in conjunction, while MacDowell & Laanes (1932) described four strains of mice with genetically different eye defects. Loeffler (1932), investigating the inheritance of an eye abnormality in the mouse in which the eyes were open at birth, found the basic gene an autosomal recessive. He considered, moreover, that one of the genes influencing its penetrance was located on the sex chromosome but since both expressivity and penetrance were so variable, the assumptions necessary rendered the demonstration somewhat inconclusive. King (1931) reported that microphthalmia in the rat behaved in outcrosses as a recessive, but was complicated in its inheritance by much overlapping with normal; selection, however, was instrumental in greatly increasing the incidence. Hain (1933) confirmed King in her finding that microphthalmia in the rat was probably recessive to its normal allele.

Lambert & Shrigley (1933) described a type of microphthalmia in the guinea-pig, variable in expression but susceptible to selection, which they tentatively concluded was due to one major gene, incompletely recessive in nature.

¹ Timoféeff-Ressovsky (1931) introduced the term "penetrance" to denote the relative frequency with which individuals, of a genetic constitution such that a given mutation is expected to appear, actually manifest that mutation; and the term "expressivity" to denote the degree to which the phenotypic expression of a mutation deviates from normality (wild type).

Among eye defects of which the inheritance is obscure are those recorded by Stockard & Papanicolaou (1916) in descendants of alcoholized guinea-pig. Guyer & Smith (1918) depicted a series of eye abnormalities in rabbits following injections of lens-sensitized fowl sera into their ancestors. The inheritance of these supposedly induced effects was highly irregular. Davis & Smith (1930) reported spontaneous coloboma in rabbits which likewise was transmitted irregularly. These authors concluded that the eye abnormalities of Guyer & Smith were due not to lens injections but to hereditary defects already present in the strain.

The peculiar embryology of the eye seems to make that organ particularly susceptible to any factor, whether genetic or environmental, tending to affect its synchronization of development. Thus it is probable that a mutation in any one of many genes regulating rate of growth could produce a defective eye, so homology of the various abnormalities in the several rodents is somewhat unlikely.

In addition to these eye defects, Keeler (1927) has recorded a type of blindness in the mouse which he descriptively termed "rodless retina", the inheritance of which is that of a simple recessive.

Ear abnormalities seem less frequent than those of the eye in rodents. Probably the most definite mutation of this class is the short ear character of the mouse, reported by Lynch (1921), which depends for its expression on a point mutation closely linked with the gene for dilute pigmentation. The character is completely recessive with no normal overlapping. No mutation even remotely comparable has been reported in other rodents although Lush (1930) described the mutation "earlessness" (very short ears) in sheep behaving as an incomplete recessive.

Two other hereditary ear defects have been noted in mice: a retarded development of the pinna (Feldman, 1932), reported to be recessive, and hound ear (McPheters & Little, 1933) which in general is also recessive but with considerable asymmetry and overlapping with the normal condition. Nordby (1930) described a congenital ear defect in swine, irregular in inheritance, which phenotypically bears a close resemblance to the hound ear of the mouse.

An odd Mendelizing character in the mouse, the complete absence of the corpus callosum, has been shown by Keeler (1933) to be due to a single recessive gene.

IV. MUTATIONS AFFECTING PELAGE

There exists among rodents a multiplicity of mutations affecting the character of the pelage of which the most striking are probably the several types of hypotrichosis. Of these, two persisting forms are found in the mouse: the dominant semi-lethal "naked" and the completely recessive "hairless"; each is conditioned by a single gene difference but they differ markedly in expression. The dominant type as described by David (1932*a*) is caused by a hypokeratosis of the hairs in consequence of which they break off; the hairs then grow out again and repeat the cycle so that a heterozygous mouse exhibiting dominant hairlessness is characterized by a succession of waves of haired and naked regions, with a resulting half-naked appearance.

Recessive hairlessness, described by Crew & Mirskaia (1931), is caused by the hairs falling out on completion of growth, beginning when the mouse is about 14 days of age, because of a malformed club ending. This character is linked with recessive spotting (Snell, 1931) with less than 10 per cent crossing-over.

A recessive hypotrichosis in the rat has been twice reported: by Roberts (1924) and by Wilder *et al.* (1932). Feldman (1935*a*) has shown that both cases are independent mutations of the same gene. Hairlessness is caused, as in the mouse, by the breaking off of individual hairs because of malformed club endings. Furthermore, rats homozygous for the recessive gene began to lose their hair at about 18 days of age, roughly at the age at which the gene for recessive hairlessness operates in the mouse. Hairless rats further resemble hairless mice in that females are often sterile while fertility of the males is unimpaired. There can be little doubt that recessive hairlessness is homologous in the two murids for the criteria of homology are completely satisfied with the exception of linkage relationships. Unfortunately, as yet no recessive spotting quite comparable with that of the mouse has been studied genetically in the rat since, phenotypically at least, hooded is clearly distinct from ordinary piebaldness.

Another character in the mouse, described by Loeffler (1934) and designated by him "hypotrichosis juvenilis", consists of the absence or under-development of the first coat of hair. Like hairlessness, it is a simple autosomal recessive but differs in that the hypotrichosis is restricted to juvenile stages while fertility and viability are unaffected.

Kislovsky (1928) and Castle (1933) have described a sublethal recessive hairless condition in the rabbit due to a partial agenesis of the follicles (David, 1932*a*). Castle has shown that the gene responsible is not borne on the same chromosome with the gene for English spotting (closely linked with recessive Dutch spotting), thus affording additional evidence that the hairless gene in the rabbit is analogous, rather than homologous, to those of the mouse and rat.

Hairlessness has also been recorded in *Peromyscus* (Sumner, 1924) in which it closely resembles that of the mouse, as well as in swine (Roberts & Carroll, 1931), sheep (Popova-Wassina, 1931), dogs and cattle.

The opposite extreme from hypotrichosis is the long-haired or angora pelage, at present existing among rodents only in the guinea-pig and rabbit, although Cocks (1852) described an angora mouse caught in England. This mutation, however, seems not to have reappeared. In both guinea-pig and rabbit, angora is a unit character recessive. This condition is also known in cats.

A frequently recurring variation of the normal pelage is the reduction or absence of the guard hairs with a resulting short, soft, plush-like, and often wavy, condition of the fur. This mutation, first encountered in the rabbit, was designated "rex", while Keeler (1935) applied the term "rexoid" to somewhat comparable mutations in the mouse.

Castle & Nachtsheim (1933) reported on the linkage relationships of the three genes for rex coat in the rabbit. These three mutations are phenotypically indistinguishable and each is conditioned by a single recessive gene. Two of the rex

genes are located on the same chromosome, with 10 or 12 per cent crossing-over, but the third is independent. These show definitely that recessive mutations in at least three normal genes produce identical effects on the pelage of the rabbit.

King & Castle (1935) studied the linkage of two dominant "curly" genes in the rat, which, although independent, produced a like somatic effect. One was found to be on the chromosome carrying the recessive gene for brown coat colour. Feldman (1935*b*) found a recessive curly hair mutation which proved not to be linked with brown although closely resembling dominant curly phenotypically.

A similar recessive mutation "waved" was recorded by Crew (1933) in the mouse. Later, Keeler (1935) reported a second "waved", phenotypically indistinguishable from that described by Crew, likewise recessive, but with the conditioning gene on another chromosome. Since the rexoid coat character follows mutations in so many normal genes there is no evidence for homology of the genes responsible in the three species of rodents.

That a similar variation has arisen in other mammals is shown by the dominant woolly hair mutation of swine, reported by Rhoad (1934). Hamilton (1934), moreover, recorded the capture of two woodchucks (*Marmota monax*) in which the long hairs were lacking, giving the animals a woolly appearance.

Instead of the smooth pelage characteristic of rodents, the hair may be rough or rosetted, radiating from centres in various parts of the body. In the guinea-pig, one such mutation is due to a dominant gene, the expressivity of which is influenced by modifying factors. In no other rodent has this mutation been reported, although Nordby (1932) described whorls in the hair of swine, perhaps somewhat comparable, which were determined by two interacting dominant genes.

V. TAIL MUTATIONS

Brachyuric or tailless mutations have been observed in mice and rats among the rodents. In the former, as Dobrovolskaia-Zavadskaia (1934) has demonstrated, the short-tailed character behaves as a dominant, lethal when homozygous. The exact degree of development of the tail is influenced greatly by accessory genes. Dunn (1934) has reported a recessive mutation, "shaker-short", in mice which closely simulates the dominant brachyury in its effect on the tail but, in addition, has an effect on the animal like that of the behaviour character, shaker.

King (1931), and Hutt & Mydland (1932), observed taillessness in rats but neither investigation recovered the character among the offspring of affected individuals. The variation appears to have been non-genetic or due to the interaction of several genes.

The short-tailed condition is characteristic of certain breeds of cats and dogs, while an extremely abbreviated tail is, of course, normally found in rabbits, as is complete external taillessness in guinea-pigs.

Hunt & Permar (1928) investigated a tail defect in mice which they descriptively called "flexed tail". This behaved in inheritance as a recessive complicated by normal overlapping. Anaemia in the new-born animal, however, was later shown

invariably to be associated with the flexed tail character (Clark, 1934), and in this manifestation the penetrance of the gene appeared to be 100 per cent. Perhaps wry tail in cattle (Atkeson & Warren, 1935) and kinky tail in swine (Nordby, 1934) are roughly analogous.

A dominant flexed tail mutation has been discovered in the mouse by Keeler (Clark, 1934) which phenotypically resembles recessive flexed except that no anaemia is associated with it. In some animals the tail is short, closely simulating brachyury. The dominant flexed gene, however, seems not to be lethal when homozygous and is believed, moreover, to be a possible lower allele of brachyury.

VI. MUTATIONS OF THE FEET

Polydactyly is probably the most striking of the mutations affecting the feet in rodents, not only because of the manner in which the normal foot is transformed but also in the way in which the defect is inherited. The types of polydactyly most thoroughly analysed genetically have arisen in the guinea-pig. Wright (1934*a*, *b*; 1935) has shown the extreme complexity of inheritance, demonstrating the genetic independence of forms appearing alike phenotypically. There seem to be at least four types, of which a rare form consists of the duplication of digits, a second which is complicated in its inheritance by environmental factors and physiologic thresholds, and a third which is dominant over the normal condition, producing polydactylous feet when the gene is heterozygous and a non-viable monster when homozygous. In addition, another type has been recorded (Castle, 1906), apparently even more dependent than the others on modifying genes, but in which the penetrance attained to 100 per cent after selection. In general, all forms vary in expressivity and most also in penetrance. With the exception of the first class of polydactylism, lost toes are replaced (the guinea-pig normally lacks the pollex on the fore foot and the hallux and fifth toe on the hind) instead of merely duplicated. The hind feet are chiefly affected, although in at least one type the influence of the genes extends to the fore feet as well.

Murray (1932) has reported polydactylous mice in which the normally pentadactyl hind foot possesses six or rarely seven toes. The extra toes occur only on the hind feet but in agreement with the situation in the guinea-pig may appear on the right, left or both feet. Since the ancestral foot presumably was pentadactyl, and judging from the anatomy of affected feet, polydactylism in mice consists of duplication of an existing toe, usually if not always the hallux. As in the guinea-pig, the expressivity varies greatly while the inheritance is extremely irregular, no one gene being of preponderant importance. It seems improbable that polydactyly in the mouse can be homologized genetically with any of the forms reported in the guinea-pig.

Polydactyly is of common occurrence in cats and has been recorded in swine (Hughes, 1935) and cattle (Roberts, 1921) as well. The posterior duplication mice of Danforth (1930) illustrate a still more radical tendency towards duplication of parts. The heredity of this abnormality is very irregular.

Little & Bagg (1924) described a defective foot mutation occurring among descendants of X-rayed mice. This abnormality varies in degree and expression and may resemble club foot, loss of toes, incomplete development, or syndactyly. The mutation, behaving as a Mendelian recessive with normal overlapping, is due to the same primary gene as that producing eye defects, acting, however, in conjunction with different modifiers. Little (1931) was able to show the efficacy of selection not only in localizing the effect to the feet but in determining the foot to which the gene restricted its action. Nothing comparable has been recorded in other rodents although syndactyly has been reported in the cat (Hays, 1917) and in swine (Detlefsen & Carmichael, 1921).

Greene (1935) described brachydactylia in the rabbit, inherited as a simple recessive, in which the phenotypic expression ranges from shortening or loss of the terminal phalanges to complete absence of the entire foot. In general, moreover, the brachydactylous condition is associated with lowered vitality.

VII. SUPERNUMERARY MAMMAE

Sollas (1909) investigating the inheritance of supernumerary mammae, a very variable character, found the hereditary behaviour irregular. It could not have been that of a simple recessive nor a simple dominant. In sheep, however, Wassin (1931) decided that extra nipples were basically conditioned by one dominant gene, while Nachtsheim (1924) demonstrated supernumerary mammae to be inherited in swine, but not simply. Hamilton (1934) has discovered examples of supernumerary mammae among woodchucks.

VIII. MUTATIONS OF THE VISCERA

Snell (1935) found an irregular dominant mutation, causing a narrowing and constriction of the spleen, together with a reduction in viability and stunting of the animal as a whole, among the descendants of X-rayed mice.

Bagg (1925), investigating the congenital absence or malformation of one or both kidneys in mice, the ancestors of which had been exposed to X-rays, found the condition closely associated in inheritance with defects of limbs and eyes, and considered them all as manifestations of a general tendency to disturbances in embryonic development. Each type, however, was susceptible to selection, indicating that localizing modifying factors were effective in determining the site of the abnormality.

IX. MUTATIONS OF BEHAVIOUR

The "waltzing" condition in which the animal manifests an inability to remain oriented, with, in consequence, a tendency to whirl in small circles, especially when excited, has long been known in mice, in which it is a unifactorial recessive. Bonhote (1912) reported that waltzing in the black rat (*Rattus rattus*) was similarly inherited as a Mendelian recessive but appeared to be more deleterious in its general effects. It has been concluded that waltzing, in the mouse at least, is determined by malformation of the middle ear. Were waltzing in both rat and mouse found to have a common structural basis the evidence of homology would be strengthened.

Cole & Steele (1922) noted the waltzing behaviour in rabbits, which, however, was more variable in its expression than is usual among mice. The condition seemed more or less hereditary but not always in the same form.

A nervous instability of somewhat the same category as waltzing was reported in the mouse by Lord & Gates (1929) and called "shaker" because of the characteristic movements of the head. When excited, the mice occasionally tend to whirl in circles, but no difficulty is experienced in distinguishing shaker mice from waltzers. Grüneberg (1935) has shown that the recessive gene conditioning shaker is located on the chromosome carrying the genes for albinism and pink-eye in the order **sh, c, p**.

Dobrovolskaia-Zavadskaia (1928) recorded a second recessive shaker gene, indistinguishable in its effects from **sh**, but independently inherited.

Although two shaker mutations have appeared in the mouse, none has been reported in any other rodent. However, another nervous condition, loosely termed epilepsy or palsy, appears sporadically among mice, although nothing is known as to its inheritance, while Cole & Ibsen (1920) determined that a comparable condition in guinea-pigs was inherited as a simple recessive. The affected guinea-pigs usually die within 2 weeks of birth, but longevity of epileptic mice seems unimpaired. Palsy in both rodents should perhaps properly be designated as tetany. Attacks are usually induced by fright, sharp sounds, etc. Lush (1930) described "nervous goats" afflicted with a similar hereditary malady.

Laanes & MacDowell (1934) have described "circling", a behaviour character in mice, as dependent upon the combined action of two recessive genes.

X. OTHER MORPHOLOGICAL MUTATIONS

Among other morphological mutations may be cited parted frontals in mice (Keeler, 1930), extra ribs and vertebrae in rabbits (Sawin, 1935), the blood groups of rabbits (Keeler & Castle, 1934), hyper- and hypoglycaemia in mice (Cambridge & Howard, 1930), etc., which have no counterparts in other rodents, or for which the inheritance has not been fully worked out.

XI. CONCLUSIONS

Although some fifty or more morphological mutations, for most of which the manner of inheritance is known, have been recognized in rodents, instances of undoubted gene homology are very rare. Recessive hairlessness in the rat and mouse and the dwarf mutation in the same two species are perhaps the most probable examples. Gene homologies, of course, may be present elsewhere but not demonstrable with the information available. Detailed developmental studies of the manner in which the gene produces its somatic effect, such as those of Bonnevie (1931) on the Little & Bagg abnormal-footed mice, Chesley (1935) on brachyury, Kamenoff (1935) on flexed tail, and David (1932*b*) on the several types of hypotrichosis, aid in bridging the gaps in our knowledge of the working of genes and supply additional information for determining gene homologies.

In comparison with colour genes, the paucity of multiple alleles in morphological mutations is noteworthy. This may indicate the relatively large number of normal genes involved in morphogenesis as compared with pigment formation, for, if mutations occurred at random among many genes, in comparatively few instances would any given one mutate two or more times.

It may be, moreover, that the gene changes basic to the relatively trivial structural mutations produce the maximum effects compatible with continued life of the zygote; weaker alleles of the conditioning gene would often have no phenotypic effect while stronger alleles would be lethal. Then, too, as Sinnott & Dunn (1935) have pointed out, genes conditioning certain morphological abnormalities are probably general in their action but occur at such a time that one region or organ in an especially critical stage in development is chiefly affected, a possibility augmenting the difficulty of determining true gene homologies. Many morphological recessives which overlap with the normal condition suggest that when penetrance is influenced by non-genetic factors, an "all-or-none" threshold reaction may be involved. With penetrance incomplete and expressivity variable, any multiple alleles which might be present could be detected only with difficulty, if at all.

Of the scores of colour and morphological mutations investigated genetically in rodents, it is strange that no undoubted example of sex-linked inheritance has been reported. This state of affairs may be partly the result of the comparatively large numbers of rodent chromosomes but it further suggests that crossing-over may take place between the *X* and *Y*-chromosomes, thus masking any sex-linked inheritance present.

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DIE ROTFÄRBUNG VON HOCHGEBIRGSSEE- ORGANISMEN

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I. EINLEITUNG

SCHON in zahlreichen Arbeiten aus den letzten Jahrzehnten des verflossenen Jahrhunderts wird die Erscheinung besprochen, dass viele Tiere der Hochgebirgseen, speziell aber die Kopepoden, rotgefärbt sind. Gelegentlich wird auch von den betreffenden Autoren—z. B. von Blanchard, Burckhardt—die Frage nach der Ursache dieser Erscheinung aufgeworfen. Eine zusammenfassende Darstellung der damals über diese Angelegenheit vorliegenden Beobachtungen und Meinungen gab Zschokke (1900). In diesem Werke stellt Zschokke die Gründe zusammen, die dafür sprechen, dass die so auffallende Rotfärbung durch die Kälte der Wohngewässer hervorgerufen werde. Zur selben Zeit beobachtete ich diese Erscheinung an den Planktonkrebsen des Achensees in Tirol und kam auf die Vermutung, dass diese Rotfärbung dazu bestimmt sei, Licht in Wärme umzusetzen. Ich verweise diesbezüglich auf meine Arbeit (1902) (S. 34 bis 40). Seither wurden gleichartige Beobachtungen noch oft an in- und ausländischem Material gemacht und die Zahl der über die Bedeutung dieser Erscheinung geäußerten Meinungen ist fast ebenso gross, wie die Zahl der Autoren, die sich mit der Sache befassten. Leider fehlt allen diesen Meinungen die experimentelle Begründung, so dass es eigentlich überflüssig scheinen möchte, wenn zusammenhängend hierüber referiert wird. Es geschieht dies lediglich aus dem Grunde, um die Aufmerksamkeit von Chemikern und Physiologen auf eine Angelegenheit zu lenken, die bisher fast nur von Tiergeographen, Morphologen und Systematikern behandelt wurde, woran vielleicht zum Teil die Schuld liegt, dass das ganze Kapitel aus dem Stadium der Hypothesenbildung noch nicht herausgekommen ist. Um aus diesem Labyrinth hypothetischer

Ansichten herauszufinden, wäre es eben nötig, dass physiologisch geschulte Biologen sich der Untersuchung des im Folgenden zu behandelnden Phänomens widmen würden, wozu eben dieser Bericht die Anregung geben will.

Bevor ich mich unserem Thema zuwende, möchte ich noch erwähnen, dass es mir seinerzeit entgangen war, dass schon vor mir* von botanischer Seite der gleiche Gedankengang eingeschlagen worden war. Kerner (1891) nämlich hat die Vermutung ausgesprochen, dass das Anthokyan Licht in Wärme umsetzen könne. Darauf wurde ich aufmerksam durch die Arbeit von Bitter (1905), in der die Widerstandsfähigkeit der anthokyangefärbten Rassen dichroitischer Pflanzen gegenüber der Kälte behandelt wird. Es gibt dazu noch andere botanische Analoga, z. B. die in Kalifornien heimische Pirolacee *Sarcodes sanguinea*, deren intensiv rote, an *Monotropa* erinnernde Blütenprosse sich aus dem Schnee heraus entwickeln; doch wollen wir uns nicht weiter mit botanischen Fällen aufhalten, auf die ich ja nur zu sprechen kam, um meine vermeintliche Priorität in dieser Sache bei Seite zu schieben, da gelegentlich in der Literatur immer noch ich als der Urheber dieses Gedankens genannt werde, so von Pesta (1930), der auf Seite 105 seines Werkes sagt: "...gibt als erster Brehm einen Erklärungsversuch..." Auch aus dem Grund bin ich hier auf meine eigene Angelegenheit nochmals näher eingegangen, weil—obgleich ich selber diesen Erklärungsversuch für zum mindesten unbewiesen, aber sogar für wahrscheinlich unrichtig halte—in der Literatur sich immer wieder zustimmende Äusserungen finden. Schon bald nach dem Erscheinen der Achen-seearbeit hatte sich Wesenberg-Lund (1904, S. 202) meiner Ansicht angeschlossen und blieb bei dieser Auffassung auch in seiner später erschienenen Arbeit (1909), in welcher er auf S. 429 sagt: "Brehm supposes that the red colouring of alpine organisms is a means of protection against the cold and gives good reasons for this supposition." Aber selbst in ganz neuen Arbeiten stossen wir auf zustimmende Äusserungen. So macht Peus unter Hinweis auf den von mir beobachteten Fall einer Rotfärbung bei *Cyclops crassicaudis* Sars die Kälte für die Rotfärbung gewisser Moortiere verantwortlich, schreibt aber diese Hypothese irrtümlich dem Zoologen Herr zu und Suchlandt (1935) sagt: "Dass die Copepoden im Davoser See die Möglichkeit haben, diese Strahlen in Wärme umzusetzen, ist eine naheliegende Vermutung."

II. WO WURDE DAS PHÄNOMEN DER ROTFÄRBUNG BEOBACHTET?

Wie schon aus den einleitenden Zeilen hervorgeht, liegen die ersten Beobachtungen aus den Westalpen und spätere aus den Ostalpen vor. Dass aber auch aussereuropäische Hochgebirge das gleiche Bild zeigen ergibt sich z. B. aus dem Vorkommen roter *Pedaliu*-exemplare in dem 2200 m. hoch gelegenen Sarry Göll in Kleinasien (Brehm, 1907), aus brieflichen Mitteilungen, die dem Verfasser von E. Hutchinson über die Fauna der Hochgebirgsseen in Tibet zukamen, von wo der Genannte schreibt: "When one sees these animals and the bright

* "Wer kann was Dummes, wer was Kluges denken
das nicht die Vorwelt schon gedacht?"

Goethes *Faust*.

red Copepoda swimming in the turquoise waters of the west Tibetan lakes, the effect is very striking." Mit dem Ausdruck "these animals" sind nahezu schwarze Daphniden gemeint. Dass dabei die tiefe Temperatur dieser Gewässer der ausschlaggebende Faktor sei, wird weiters nahegelegt dadurch, dass ausser den Hochgebirgen besonders die arktischen und antarktischen Regionen Gewässer mit rotgefärbten Organismen aufweisen. Mir selbst fiel dies besonders an dem Kopepoden *Eurytemora Raboti* de Guerne auf, der mir aus Seen von Spitzbergen vorlag, sowie an dem Ostracoden *Herpetocypris glacialis* Sars aus Grönland. Im antarktischen Gebiet sind es besonders die zu den Copepoden gehörigen Boeckelliden, die sich durch intensives Colorit auszeichnen und zwar im südlichsten Teil von Südamerika in Tieflandseen weiter nordwärts in den Hochseen der Cordilleren. Dort hat speziell Prof. Rahm Beobachtungen angestellt, über die er berichtet: "In allen Cordillerenseen von Mittelchile bis Feuerland, in Seen, deren Temperatur durchschnittlich nicht 10 Grad übersteigt fand sich die Rotfärbung, die aber nach wenigen Tagen verschwand, wenn die Tiere im Tiefland in Aquarien gehalten wurden." Eine von diesen Formen habe ich kürzlich eben mit Rücksicht auf ihre Färbung als *Pseudoboeckella erubescens* beschrieben. Ebenfalls für die Annahme einer Kältewirkung könnte schliesslich noch das Vorkommen der roten Hydren im kalten Tiefenwasser vieler Seen sprechen, oder das Vorkommen grellroter Milben im kalten Wasser der Gebirgsbäche, doch scheint in diesen Fällen etwas von den früheren Beispielen prinzipiell Abweichendes vorzuliegen.

Ein weiteres Argument, das auch dafür spricht in der Kälte die Ursache der Rotfärbung zu sehen, ist ferner der schon von mir in der Achenseearbeit erwähnte Umstand, dass die Schneeflora reich an roten Organismen ist. Vor allem ist es die Gattung *Chlamydomonas*, die weite Schneeflächen rot färbt, gewöhnlich die Art *nivalis*, in Norwegen auch *alpina* und auf den südamerikanischen Anden *sanguinea*, *tingens* und andere Formen. Auch eine *Gloeocapsa*, nämlich *sanguinea*, kann Ursache rosenroter Schneefarbe sein und in selteneren Fällen Peridineen, wie *Gleodinium Pascheri*, das von Suchlandt (1935) von Davoser Schneefeldern beschrieben wurde und *Peridinium cordis Mariae* das im Manuskript von Fräulein Traunsteiner als den Schnee bei Kitzbühel in Tyrol rotfärbende Art beschrieben wurde.

III. WELCHE ORGANISMEN KOMMEN ALS TRÄGER DER ROTFÄRBUNG IN BETRACHT?

Aus den vorangehenden Zeilen scheint hervorzugehen, dass die Kopepoden fast ausschliesslich die rote Hochgebirgsseefauna zusammensetzen. Dies bedarf aber doch noch einer gewissen Einschränkung, weshalb wir hier der Reihe nach noch die anderen Komponenten dieser Fauna anführen wollen. Ferner möge dabei auf Angehörige derselben Tiergruppen aufmerksam gemacht werden, die ebenfalls lebhaftige Färbung zeigen, ohne indes Kaltwasserbewohner zu sein. Durch diese Zusätze möge eine vorschnelle Herstellung kausaler Beziehungen zwischen Farbe und Milieu vermieden und vielleicht künftigen Untersuchungen ein Fingerzeig gegeben sein, wo die kausalen Zusammenhänge zu suchen seien.

(1) *Kopepoden*. Wenn wir die Diaptomiden unserer Alpenseen überblicken, so finden wir, dass keine erhebliche oder nur eine schwache im Winter eintretende Rotfärbung bei den Arten *gracilis*, *graciloides*, *laciniatus* vorkommt, die in den tiefer gelegenen Seen leben, während in den Hochgebirgsseen zwei meist lebhaft rotgefärbte Arten auftreten: *denticornis* und *bacillifer*. Eine eigentümliche Stellung nimmt die Art *tatricus* ein. Auch sie lebt vor allem in hochgelegenen Gewässern, nämlich in Almtümpeln; aber die Wohngewässer des *Diaptomus tatricus* zeigen meist ziemlich hohe Temperaturen, zum mindesten tagsüber, so dass ihre Bewohner nicht gut als Kaltwasserformen betrachtet werden können. Das gleiche Verhalten zeigen nun aber auch gewisse *bacillifer*-Kolonieen. *Diaptomus bacillifer* gehört zu jenen eigentümlichen Organismen, die zwei extrem verschiedene Wohnorte besiedeln können, nämlich Hochgebirgsseen und oft überhitzte Kleingewässer des Tieflandes. Beispiele für den oben erwähnten Fall, dass auch in warmen Gewässern lebhaft gefärbte Diaptomiden auftreten können bieten die schon durch ihren Namen als lebhaft pigmentiert gekennzeichneten Species *superbus*, *cyaneus*, ferner *amblyodon* oder der südamerikanische *granulosus*. Man könnte daraus den Schluss ziehen, dass zufällig von Haus aus lebhaft gefärbte Arten sich geeignet erwiesen, Hochgebirgsseen zu besiedeln oder dass dem Ausnützungsprinzip von Becher entsprechend schon von vornherein gefärbte Arten ins Hochgebirge eindringen, weil ihre Farbe sie für den Aufenthalt dortselbst besonders geeignet machte. Es wäre also in diesem Falle die Färbung nicht Folge, sondern Voraussetzung des Lebens in kalten Gewässern. Aber es verdient der Umstand Beachtung, dass die aus dem Gebirge in Zimmeraquarien versetzten Tiere ausbleichen, wie besonders die von Rahm (1932) in Südamerika gemachten Versuche zeigten. Unter den Cyclopiden wurde besonders für die Arten *strenuus* und *serrulatus* von vielen Autoren die Rotfärbung im Hochgebirge vermerkt. Für *serrulatus* konnte ich nicht nur Rotfärbung in den hochgelegenen Pfitscher-Joch-Seen in Tirol nachweisen, sondern auch Rotfärbung bei Tieflandkolonieen während des Winters. Unter den Harpacticiden wieder fand ich besonders bei *Canthocamptus rhaeticus* Schmeil öfters Rotfärbung in Gebirgsseen. Im übrigen aber fällt die Erscheinung bei dieser Kopepodengruppe weniger auf und folgender Fall scheint sich überhaupt nicht gut mit der Annahme der Kältewirkung in Einklang bringen zu lassen. Im Lunzer Untersee finden wir mehrere Harpacticidenarten im durchwärmten Uferwasser, die alle keine besondere Färbung aufweisen. In einer bei etwa 12 m. gelegenen Tiefenzone, die durch das Auftreten zahlreicher roter Organismen ausgezeichnet ist, lebt *Canthocamptus northumbicus* Brady in grellroten Exemplaren. Da in dieser Tiefe schon konstant kaltes Wasser vorliegt, könnte man denken, hier wäre ein Beispiel dafür gegeben, dass das wenige kurzwellige Licht, das in diese Zone noch eindringt, in Wärme umgesetzt wird. Aber gleich unterhalb dieser Zone, beginnt dann eine Region, die von einigen anderen *Canthocamptus*-arten, besonders von *C. Wierzejskii* Mraz. bewohnt wird und alle diese Arten sind wieder farblos. Nun könnte man ja glauben, dass hier eben bereits das Licht fehle, das in Wärme umgesetzt werden solle; da aber in der gleichen Zone noch assimilierender *Campylodiscus* vorkommt, wird diese Annahme recht zweifelhaft, was zur Folge hatte, dass

ich zu der Meinung verleitet wurde, die Rotfärbung des *Canthocamptus northumbicus* rühre davon her, dass er die roten Mikroorganismen der von ihm bewohnten Zone als Nahrung aufnehme und dieser Nahrung seine Farbe verdanke. Dass auch diese Meinung irrtümlich ist, wird uns weiter unten noch beschäftigen.

(2) *Cladoceren*. Hier kommen vor allem die pelagischen Daphnien und Bosminen in Betracht. Ferner wäre erwähnenswert, dass *Alonopsis elongata* Sars in einer eigenen sepiabraunen Rasse in Kaltwasserseen auftritt. Auch hier finden wir, ähnlich wie bei den Kopepoden, lebhaft gefärbte Arten in wärmeren Gewässern, etwa *Latona setifera* O.F.M., deren Farbenpracht Weismann veranlasste, sie auf einer kolorierten Tafel abzubilden. Die Farbe von *Latona* oder *Alonopsis* ist aber, wie sich gleich zeigen wird, prinzipiell von der Färbung der Daphnien und Bosminen verschieden.

(3) Die *Rädertiere*, die neben den eben erwähnten Kleinkrebsen den Hauptbestandteil des Planktons unserer Süßwasserseen bilden, nehmen an dem Phänomen der Rotfärbung so gut wie gar keinen Anteil. Neben den grellgefärbten Krustern treten sie in farblosen Individuen auf. Zwar konnte eingangs die Rotfärbung des *Pedalion fennicum* aus dem Sarry Göll in Kleinasien erwähnt werden, aber das *Pedalion bulgaricum* aus Hochgebirgsseen in den Hohen Tauern fand ich farblos.

(4) Und ähnlich liegen nun die Verhältnisse bei den übrigen Tiergruppen. Noch nie scheint ein roter Nematode im Hochgebirge beobachtet worden zu sein, von Hydracarinern wurden zwar eingangs Fälle erwähnt, aber sie verlieren an Beweiskraft, da sie Familien angehören, die überhaupt durch den Besitz roten Pigmentes ausgezeichnet zu sein scheinen u.s.w.*

J. Kühtreiber (1934, S. 13) sagt: "Anschliessend sei noch bemerkt, dass überhaupt typisch alpine Arten eine deutliche Neigung zur Rotfärbung zeigen. So werden z. B. *Perlodes intricata* und *Rhabdiopteryx alpina* durch prachtvoll orange-gelbe Fleckung ausgezeichnet. Die rotgelbe Färbung tritt neben der arttypischen Zeichnung auch an allen schwächer chitinierten Stellen auf, z. B. an der Pronotumeinfassung, gewissen Partien des Abdomens der Thorakalpleuren, u.s.w. Die Erscheinung der Rotfärbung alpiner Arten wurde bereits von Friese und Wagner (1904) für Hummeln nachgewiesen."

Darnach scheint also das Phänomen der Rotfärbung im Hochgebirge auch bei der Landfauna vorzukommen. Doch spielt es bei dieser ohne Zweifel nicht eine so auffallende Rolle wie bei den Wasserbewohnern.

Das Uneinheitliche, das sich in diesem Verhalten zeigt, lässt bereits vermuten, dass hier heterogenes Material vorliegt, eine Vermutung, die sich dadurch bestätigt, dass wir behaupten dürfen, dass schon die Pigmente, die hier in Frage kommen, ganz verschiedener Natur sind.

* Vielleicht aber könnte Hydra in Betracht kommen, die im kalten Tiefenwasser der Seen wie im Hochgebirge in roten Formen auftritt.

IV. WELCHE PIGMENTE VERANLASSEN DAS PHÄNOMEN DER ROTFÄRBUNG?

Es ist eine der Hauptschwierigkeiten bei der Behandlung unseres Thema, dass über die chemische Natur der Pigmente, die hier im Spiele sind, fast gar nichts bekannt ist. Gleich einer der ersten Beobachter, Blanchard (1890), hat gezeigt, dass die Rotfärbung hochalpiner Kopepoden durch karotinartige Stoffe bedingt ist, von denen er auf Grund des Absorptionsspektrums ihrer Lösungen zwei Arten unterschied, die er als Carotin im engeren Sinne und als Diaptomin unterschied. Über dieses Stadium sind aber die späteren Untersuchungen nicht hinausgekommen. Überblickt man die von verschiedenen Autoren mitgeteilten Meinungen über die Natur der in Betracht kommenden Farbstoffe, Meinungen, die—das sei nochmals betont—der chemischen Fundierung ermangeln, so ergibt sich etwa folgende bereits von Wagler (1912) vorgenommene Gruppierung:

1. Diffuse Cuticularfärbung, wie sie etwa im Falle *Alonopsis* vorliegt.
2. Färbung durch Chromatophoren der Hypodermis, wofür *Latona* ein eklatantes Beispiel bietet.
3. Fettkörperfärbungen, wozu vor allem die Karotinfärbungen der Kopepoden zu rechnen sind.
4. Pathologische, durch Parasiten hervorgerufene Färbungen.

V. DIE ÜBER URSACHE BZW. ZWECK DIESER FÄRBUNGEN AUFGESTELLTEN HYPOTHESEN

Vielleicht die älteste Deutung die vorliegt, die aber nicht eigentlich der Hochgebirgsfauna gilt, aber vielfach mit dieser konfundiert wurde, rührt von Weismann (1879) her und nimmt an, dass es sich um Schmuckfarben handle, die bei der sexuellen Zuchtwahl eine Rolle spielen. Gegen diese Annahme wendete sich Frič (1893), der darauf hinwies, dass bei *Holopedium gibberum* der Eintritt der Färbung nicht mit der Sexualperiode zusammenfalle. Nach Frič konnte ich gegen die Weismannsche Auffassung ins Treffen führen, dass im Achensee prachtvolle saphirblaue Färbung bei eine Bosminakolonie vorliegt, die die bisexuelle Vermehrung überhaupt eingebüsst hat und dass bei diesem Fall überdies der Sitz der Färbung vor allem der Körper der Embryonen sei. Über diese Schwierigkeit dürfte auch der Versuch von Scheffelt (1908) nicht hinweghelfen, der bei der acyclischen Bosminakolonie aus dem Schwarzwälder Titisee ebenfalls diese "Schmuckfarben" konstatierte und in ihnen den letzten atavistischen Rest der verschwundenen Sexualperiode erblickt.

Der nächste Versuch einer Deutung, der zugleich ausdrücklich die Fälle der Hochgebirgsfauna betrifft, ist die bereits eingangs erwähnte, von mir herrührende Deutung, dass es sich bei diesen Farben um einen Transformator handelt, der Licht in Wärme transformiert. Ganz abgesehen davon, dass dieser Annahme die experimentelle Bestätigung und die physikalische Begründung fehlt, erregte sie Widerspruch durch Fälle von Rotfärbung in Gewässern, die nicht kalt temperiert waren. Ein solcher Fall lag zunächst einmal vor in dem Auftreten der *Euglena*

sanguinea in überhitzten* Almtümpeln, die durch die Massenv egetation dieses Flagellaten den Charakter sogenannter Blutseen annehmen. Klausener (1908) suchte diesen Widerspruch dadurch aufzuklären, dass er annahm, es liege bei der erwähnten *Euglena* ein anderer Farbstoff vor, das Hämatochrom, das die Eigenschaft habe, die ultravioletten Strahlen zu absorbieren und so als ein Lichtfilter zu wirken, das die schädliche Komponente des Sonnenlichtes, die im Hochgebirge reichlich vorhandenen ultravioletten Wellen, abschirme.

Bald nachher äusserte Steiner (1911) die Meinung, die roten Farbstoffe der Hochgebirgsseetiere seien ein Atmungspigment, das bei unzulänglicher Sauerstoffversorgung die Atmung auf der erforderlichen Höhe halte; gegen diese Meinung konnte alsbald eingewendet werden, dass der hier postulierte Sauerstoffmangel in den meisten Seen der Hochgebirge gar nicht vorliege.

Schon vorher war von einigen Autoren z. B. von Wagler (1912) behauptet worden, dass die Färbung von der Ernährung abhäng e, eine Meinung die seither noch von einigen weiteren Autoren z. B. Spandl (1924) vertreten wurde, wobei die Meinungen nach zwei Richtungen auseinander gehen, indem die einen glauben, dass die lebhaft e Färbung die Begleiterscheinung eines guten Ernährungszustandes sei, dass also gewissermassen die quantitative Seite der Ernährung in Betracht käme, während andere die Qualität des Futters als Färbungsursache in Anspruch nehmen. Die Anhänger der zweiten Annahme stützen sich entweder auf Experimente, bei denen Pigmente der Nahrungsorganismen im Versuchstier in unveränderter Form gespeichert wurden, teils auf solche, bei denen aus farblosen Futterorganismen auf synthetischem Wege erst gefärbte Stoffe gebildet wurden. Es seien einige dieser Fälle hier zitiert, um zugleich zu zeigen, dass diese Beispiele für die gewöhnlichen Fälle gefärbter Tiere aus Hochseen kaum in Betracht kommen dürften.

Oft wurde die Meinung geäussert, dass die Aufnahme gefärbter Futterorganismen als Färbungsursache in Betracht komme.† Ganz abgesehen davon, dass dann wieder die Frage nach der Ursache der Färbung dieser Organismen sich erhebt, kommt noch eine zweite Schwierigkeit in Betracht. Wie schon oben erwähnt wurde, legte das Vorkommen des roten *Canthocamptus northumbicus* in der Zone der roten Organismen im Lunzer Untersee die Annahme nahe, dass seine rote Färbung davon herrühre, dass er die verschiedenen roten oder blauen Flagellaten und niederen Pflanzen dieser Zone als Nahrung aufnehme und durch diese gefärbt werde. Allein, wie Pascher (1924) betonte, werden die hier in Frage kommenden Farbstoffe im Darm der Kruster chemisch abgebaut und haben gar keine Beziehung zu der Carotinfärbung des erwähnten *Canthocamptus*, da sie Phykoerythrine sind.‡

* Eine andere rote *Euglena*, nämlich *haematodes*, ist nach Gams (1924) sogar thermophil!

† So nahm Blaas (1924) an, dass bei den von ihm beobachteten Diaptomiden das rote Diaptomin ein Abkömmling des Chlorophylls der Futteralgen sei und Lwoff (nach mündlicher Mitteilung von Prof. Bresslau. Stelle der Publikation ist mir unbekannt) kam durch seine Versuche an dem marinen Copepoden *Idya furcata* zu der Ansicht, dass deren carotinoides Pigment von der Nahrung gebildet werde.

‡ Analog sind wohl die Verhältnisse bei den roten Krebsen und Würmern, die der von *Phyllophora rubens* gebildeten Rotalgenfacies des Schwarzen Meeres angehören.

Dass andererseits sogar aus farblosen Komponenten der Nahrungsorganismen Farbstoffe aufgebaut werden können, zeigt ein Versuch mit *Hydra*, die durch Fütterung mit weissen Branchipodiden sich rot färbte. Ja selbst für Schmarotzer kennt man einen analogen Fall: Der Acanthocephale *Pomphyrhynchus* tritt in roten Exemplaren auf, wenn er in farblosem *Gammarus* parasitiert, hingegen in farblosen, wenn *Synurella* der Wirt ist. Ja sogar der Fall kommt vor, dass nicht der Parasit sondern der Wirt, durch einen farblosen Parasiten gefärbt wird. Marshal (1934) berichtet, dass marine Calanusarten durch den Trematoden *Hemiurus* "brillant scharlachrot" gefärbt werden, sowie auch durch gewisse Cestoden und Dinoflagellaten, z. B. *Syndinium*, während kein Fall bekannt wurde, in dem etwa ein Nematode eine solche Verfärbung ausgelöst hätte, wiewohl Nematoden sehr oft als Parasiten beobachtet wurden. Es ist noch fraglich, ob diese Erscheinungen wesensgleich sind mit den Beispielen, die als pathogene Färbungen beschrieben worden sind.

Hier wäre weiters der Fall zu erwähnen, dass Daphnien, deren Haemolymph mit *Spirobacterium daphniae* erfüllt ist, eine grell ziegelrote Färbung aufweisen, während mit Microsporidien infizierte Daphnien opak milchweiss werden und solche Exemplare, die von *Cysticercus mirabilis* befallen sind, sich durch eine geschwärzte Haemolymph von den gesunden Exemplaren unterscheiden. Also auch nur ein ganz bestimmter farbloser Parasit ruft Rotfärbung hervor. Natürlich kommen die Fälle parasitärer Rotfärbung für die Hochgebirgsfärbung nicht in Betracht. Sie könnten aber für die Entstehung der roten Pigmente aufschlussreich werden.

Rammner (1932) hat in einer Arbeit über Einwirkungen der Übervölkerung auf *Daphnia pulex* auf eine pathologische Rotfärbung aufmerksam gemacht, die er als Folge der durch die Übervölkerung veranlassten Degeneration der betr. Populationen auffasst. Der Fall ist deshalb merkwürdig, da Wagler (1912) ganz im Gegensatz zu Rammner zu dem Resultate kam: "Die Färbungen—sc. bei Cladoceren—sind lediglich Zeichen eines gewissen Wohlbefindens und guter Ernährung." Auch mag es auffallen, dass Kaj Berg (1934),* der in letzter Zeit viel mit übervölkerten Populationen arbeitete, um durch Depressionszustände bisexuelle Fortpflanzung auszulösen, nichts über solche Färbungen bei seinen Kolonien berichtet.

In den Arbeiten von Pesta (1930) und Steiner (1911) stossen wir auf die Annahme, dass möglicherweise Lösungen, die im Sinne der Theorie von Pütter aufgenommen wurden, zu einer Synthese der Farbstoffe führen oder dass es sich um Stoffe handle, die im Dienste einer Photosynthese stehen.

In der eingangs zitierten Achensee-arbeit (1902) habe ich die Meinung, dass die Rotfärbung Kältewirkung bzw. Kälteschutzmittel sei durch den Hinweis auf die Rotfärbung vieler Algenzygoten unterstützt, wie sie vor der Winterruhe von Algen gebildet werden. Aber ganz abgesehen davon, dass viele solche Zygoten gerade zur Zeit der Sommerruhe gebildet werden, etwa bei *Sphaeroplea annulina* wurde von Senn (1911) und Geitler (1923, 1930) gezeigt, dass diese Haemato-

* *Biological Reviews*.

chromfärbungen zumindestens in einigen Fällen die Bedeutung eines Reservestoffes haben.

Gleichzeitig zwei Ansichten wurden jüngst von Suchlandt (1935) geäußert, der zunächst für die Kopepoden annimmt, dass die roten Ölkugeln etwa die Bedeutung eines Heizkörpers haben, wie man gewissen Pigmenten, die den Tracheen verschiedener Chironomidenlarven angelagert sind, eine solche Bedeutung zugeschrieben hat. Während aber diese Tracheenpigmente, die übrigens keine Carotine sind, die Aufgabe haben sollen, die Luftcirculation in den Tracheen durch Schaffung eines Temperaturgefälles zu fördern, glaubt Suchlandt den roten Ölkugeln die Aufgabe zuweisen zu müssen, dass sie wie Linsen wirken und die Gonaden anwärmen, weshalb sie bei den Cyclopen immer in der Nachbarschaft der Gonaden lägen.

Der genannte Autor erörtert aber noch eine zweite Möglichkeit, nämlich die, dass die roten Farbstoffe als Vitaminquelle fungieren. Für die Carotine ist dies ja bereits gesichert, da sie in das antixerophthalmische Vitamin A übergeführt werden können; man kam an marinem Plankton zu dem Resultat, dass die Lipochrome des Zooplanktons zur Quelle für das genannte Vitamin werden können. Beide Annahmen Suchlandts könnten auch gut mit dem starken Hervortreten der Rotfärbung im Hochgebirge oder in den polaren Zonen in Einklang gebracht werden. Die Deutung der roten Farbe als Heizkörper wäre ja in den kalten Regionen recht plausibel. Aber diese Deutung mutet in anderer Hinsicht recht gezwungen an. Die andere Auffassung könnte wieder damit gut vereinbart werden, dass gerade im Hochgebirge starke ultraviolette Strahlung vorliegt, die ja gerade für die Synthese mehrerer Vitamine von ausschlaggebender Bedeutung ist.

Den Abschluss dieser Übersicht möge noch eine schon ältere Mitteilung von Schulze (erwähnt bei Hesse (1896)) bilden, der eine prachtvoll blau und gelb gefärbte Population von *Chirocephalus Grubei* in einem Graben beobachtete, in dem eine Sprungfederematratze verrostete und der daher der Meinung war, die Färbung der Tiere sei durch den Eisengehalt des Wassers also durch äussere chemische Einflüsse verursacht. Dem ist aber entgegenzuhalten, dass dieser Phyllopode überhaupt zur Entfaltung grosser Farbenpracht neigt, so dass pigmentarme albinotische Populationen geradezu auffallen, weshalb dieser Erscheinung Hesse (1896) einen besonderen Artikel widmete. Elster (1896) berichtet darüber, dass im Saale-Elbegebiet die *Chirocephalus Grubei* Kolonien alle prächtig gefärbt waren und zwar eine Population konstant blau, eine andere rot, eine dritte rosenrot mit grasgrünen Beinen und eine vierte umgekehrt grün mit rosenroten Beinen. Hier scheint es sich um Konstitutionsfärbungen zu handeln, die überhaupt bei Phyllopoden recht verbreitet sein dürften. Denn mir liegt eine russisch geschriebene Arbeit von Ermakow (1928) über die Fauna der südrussischen Salzseen vor, der eine Farbentafel beigegeben ist, auf der *Artemia salina* abgebildet ist und zwar das Weibchen lachsrot mit grünem Kopf und teilweise grünen Beinen, das Männchen mit grünem Körper und roten Beinen, also ganz analog dem oben erwähnten *Chirocephalus*-beispiel. Wir sehen hier also wieder den Fall auffallender Färbungen auch im Tiefland, wie wir sie schon eingangs bezüglich einiger Diaptomiden erwähnten.

In derselben Abhandlung setzt sich Elster (1896) auch mit der merkwürdigen Färbung des *Diaptomus superbis* auseinander, der in demselben Untersuchungsgebiet im März in rot gefärbten Exemplaren auftrat, die sich dann im Mai blau umfärbten*. "Männchen und Weibchen färben sich gleichzeitig unter verschiedenen Bedingungen in Blau um, offenbar liegt das im Entwicklungsgang der Tiere". Damit scheint Elster sagen zu wollen dass er der Färbung keine besondere Bedeutung beimessen möchte doch erwähnt er für *Heterocope Weismanni* aus dem Bodensee, dass die blau gefärbten Tiere an der vertikalen Wanderung nicht teilnehmen, dass also vielleicht die Färbung eine physiologische Bedeutung hätte, die in diesem Falle wieder auf einen Zusammenhang mit dem Lichte deuten würde. "Über all diese Fragen zu theoretisieren", sagt Elster in seiner Abhandlung, "dürfte zwecklos sein und hiesse den begonnenen experimentellen Untersuchungen vorgreifen." Wenn ich diese Warnung nicht beachtet und zwar nicht theoretisiert aber einen Überblick über die mannigfachen Ansichten gegeben habe, die mit der Färbung der Hochgebirgsseetiere zusammenhängen, so geschah dies z. T. deswegen, weil mir keine experimentellen Untersuchungen auf diesem Gebiete bekannt geworden sind, obwohl seit der Veröffentlichung Elsters Jahre vergangen sind.

Ordnen wir die hier mehr in chronologischer Aufeinanderfolge skizzierten Anschauungen, so zeigt sich, dass trotz der im Bereich der Biologie herrschenden Verpönung der finalen Betrachtungsweise fast alle Autoren das Phänomen der Rotfärbung unter finalen Gesichtspunkten betrachten. So durch die Annahme, die rote Farbe sei

1. Sexuelles Lockmittel.
2. Diene dem Wärmegevvinn.
3. Der Abschirmung ultravioletter Strahlung.
4. Der leichteren Gewinnung des Sauerstoffes.
5. Sie bezwecke eine Photosynthese.
6. Eine Heizung der reifenden Gonaden.
7. Die Entstehung von Vitaminen.
8. Sie diene der Ernährung als Reservestoff.

Kausal zeigt sich die Betrachtungsweise nur in drei Fällen, nämlich, wenn man annimmt, die Farbe werde hervorgerufen

9. Durch die aufgenommene Nahrung.
10. Durch den Chemismus des Wohngewässers.
11. Durch die Konstitution, d. h. sie liege in der Reaktionsnorm der betreffenden Art und habe keine physiologische Bedeutung.

Welche von diesen Auffassungen und ob überhaupt eine von ihnen zutrifft, das wird erst entschieden werden können, wenn über die Natur der zugrunde liegenden Farbstoffe Untersuchungen vorliegen werden, die über eine lediglich spektroskopische Unterscheidung der fraglichen Pigmente hinausgehen. Um aus dem Irrgarten der von Hydrobiologen aufgestellten Vermutungen und Hypothesen

* Hier mochte ich daran erinnern, dass grellrot gefärbter *Diaptomus denticornis* Wierz., den ich im Misurina-See in den Dolomiten fischte lebhaft blau gefärbt wurde, als ich dem Fang Formaldehyd zusetzte.

herauszufinden, hat vorerst einmal der Chemiker und der Physiologe das Wort. Untersuchungen von dieser Seite anzuregen ist der Zweck der vorliegenden Zusammenstellung.

VI. ZUSAMMENFASSUNG

In Hochgebirgsseen treten auf der ganzen Erde wie auch in den Gewässern der Polarzone bei bestimmten Tiergruppen rote Pigmente auf, über deren Entstehung und Bedeutung im Laufe der letzten 50 Jahre verschiedene Ansichten geäußert wurden. Die elf hierüber aufgestellten Hypothesen werden in konkreten Fällen angeführt und zur Discussion gestellt, da keine dieser Annahmen experimentell gestützt ist und demnach alle der Nachprüfung von chemischer und physiologischer Seite bedürfen.

VII. SUMMARY

Red pigments are found in certain groups of animals occurring in mountain lakes in all parts of the world and in polar waters. In the course of the last half century various opinions have been expressed concerning the origin and significance of this red coloration. In this article eleven hypotheses have been reviewed. None of these has an experimental basis and all therefore require to be tested chemically and physiologically.

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NACHTRAG

Durch die gütige Vermittlung meines Freundes Prof. Dr Ruttner konnte ich in zwei Publikationen Einsicht nehmen, die darüber orientieren, wie weit sich die reine Physiologie mit dem hier behandelten Thema beschäftigt hat. Im Jahre 1934 erschien in den "Monographien aus dem Gesamtgebiet der Physiologie" als Band 31 das Werk *Carotinoide* von L. Zechmeister. Zu meiner Überraschung musste ich feststellen, dass darin die Fälle von Carotinfärbung, die in unserem Bericht behandelt werden, überhaupt nicht erwähnt sind und dass, trotzdem das Register der verarbeiteten Literatur rund 700 Nummern umfasst, darin nicht die wichtigen Arbeiten von Zopf behandelt werden, über die sich eine kurze übersichtliche Darstellung in dem bekannten Werke *Vergleichende chemische Physiologie der niederen Tiere* von O. von Fürth (Jena, 1903) findet, aus der hier das wesentliche herausgegriffen sei, da nach dem Werke von Zechmeister zu urteilen, seither nichts Neues dazugekommen zu sein scheint. Nach den Untersuchungen an höheren Krebsen lägen zwei Farbstoffe vor, das rote Crustaceorubin und das blaue Cyanokrystallin, von denen das letztere ziemlich labil ist und leicht zum Teil in Crustaceorubin umgesetzt werden kann. Bei niederen Krebsen (Copepoden und Phyllopoden) konnte Zopf zwei Farbstoffe isolieren, von denen der eine, das "gelbe Carotin", zwei Absorptionsstreifen im grünblauen Teil des Spektrums zeigt, während der zweite, das "rote Carotin" oder Diaptomin, nur ein Absorptionsband aufweist. Aus dem gleichen chemischen Verhalten des roten Carotins und des Crustaceorubins schliesst Fürth auf die Identität dieser beiden Körper. Hinsichtlich der Blaufärbung niederer Krebse lag Fürth nur eine Untersuchung Colosantis über den marinen Copepoden *Anomalocera Patersoni* vor—er wird an der betr. Stelle irrtümlich als *Petersonii* bezeichnet—und Fürth vermutet, dass dessen Färbung auf Cyanokrystallin zurückzuführen ist. Nebenbei gesagt ist die Färbung dieses Copepoden auch einer der Gründe gewesen, der mich daran zweifeln liess, dass die Färbung vieler Kleinkrebse eine Kältewirkung darstelle, da *Anomalocera* im warmen Mittelmeer lebt.

PROPERTIES OF THE CELL SURFACE

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I. INTRODUCTION

It is obvious that the surface of a free-living cell may be important, (1) as a barrier determining the diffusion of substances in or out; (2) as the seat of electromotive forces, whether phase boundary, membrane or diffusion potentials; (3) as a boundary having a definite tension with resultant internally directed pressure; (4) as a protection of the cell contents from mechanical destruction. Thus have arisen the important physiological fields of permeability, bioelectric potentials and surface forces of the cell. The last subject has received less attention than the other two, in part because of difficulties in measurement.

This article will deal with the measurement of tension at the cell surface, and the properties of surfaces resulting from the existence of a layer or layers of molecules, oriented in a definite manner. Since many cells possess protective or supporting membranes it must be very clearly stated at the beginning that it is the surface of the protoplasm or the so-called plasma membrane with which we are concerned.

Distinctness and rigidity in protective layers at the surface of cells varies from the very rigid cellulose of plants, through the definite membranes of various marine eggs, to the very delicate pellicle of so-called naked cells. The membrane of naked cells is so intimate a part of the protoplasm that its separation is difficult, since its properties approach that of the plasma membrane. These protecting membranes do not interfere with the study of cell permeability since they are mostly readily permeable to all except very large molecules; nor do they interfere with the measurement of cell potentials since their electrical conductivity is high; but the tension at the surface of such cells obviously cannot be measured owing to the thick membranes.

It would be futile to draw a sharp distinction between cells with membranes and those without. All possible gradations are to be found. Although the behaviour of a solution depends on the size of its molecules, and the extremes of colloid and crystalloid are easily recognizable, an ill-defined group of semicolloids must be invented to include the intermediate categories. Likewise it is impossible to say when a surface ends and a membrane begins, and cells exist whose boundary presents some of the characteristics of an interface and some of the characteristics of a membrane.

Let us consider only (1) cells whose surface is not rigid, since such cells can undergo movement (amoeba, leucocytes), or can readily be fragmented by shaking or centrifuging (certain marine eggs), or (2) protoplasts, in which the obvious external membrane can be separated either by plasmolysis (in plants) or by micro-dissection (cf. Chambers & Höffler, 1931). Although such cells are non-miscible with their medium their surface cannot be compared with the interface between two pure non-miscible fluids, and only such a surface can be said to possess pure surface tension of a definite value. If monolayers or multilayers of a third substance are present, reproducible tensions may be measured, as in a soap film, but the value will depend on the concentration of surface-active substances in the three-dimensional phases. Finally the thickness of a third substance may reach the point where a thin film may be said to exist, and a third three-dimensional phase, however thin, is to be considered. Obviously all intergrades exist.

A true membrane may be solid. Work must be done to bend it. Evidence of its existence is frequently obtained by crushing or tearing cells, when the frayed ends of the membrane remain as rigid solid films. However, it must not be forgotten that many monolayers behave like solids and are classified as solid films.

A membrane may also be semi-solid, a liquid of such high plasticity that it retains its form against ordinary disturbing forces, but moves under greater ones. Such appears to be the condition of the pellicle of unfertilized *Arbacia* and *Asterias* eggs, which can be moved by centrifugal force in the centrifugal direction (Harvey, E. B., 1932). On fertilization the "pellicle" is elevated and hardens to form the fertilization membrane. In centrifuged eggs on fertilization a fertilization membrane appears only at the heavy or centrifugal end (Costello, 1935), and micro-dissection shows that a pellicle is no longer present at the centripetal end (Chambers, 1935; see Hobson, 1932).

A solid or semi-solid membrane must possess elasticity. Its tension will increase as it is stretched, whereas it is characteristic of a surface showing a pure surface tension that the value of this tension is independent of the extension of the surface. A plot of tension against area of surface gives a straight line parallel to the area axis, as in the case of a soap bubble, where the tension (T) is easily calculated from the internal pressure (P) and radius (r), $T = Pr/4$.

On the other hand a surface film only one molecule thick may possess elasticity. This is true of protein films at both the air-water interface (Hughes *et al.* 1932) and at the oil-water interface (Askew & Danielli, unpublished). Harvey & Danielli (1936) showed that bubbles of egg albumen in air have a small but definite elasticity. Albumen bubbles show hysteresis; the tension-pressure curve follows a different course during inflation and deflation of the bubble. Thus it is not surprising that the cell surface, as Cole (1932) has found, also possesses elastic properties. It would be useless to quibble over the terms film, pellicle, membrane, etc. and it is sufficient for our purpose to remember that what is measured as the tension at the surface of cells is the sum of the surface and elastic tensions of a definite molecular structure.

II. MEASUREMENT OF SURFACE FORCES IN CELLS

Only those cells will be considered whose surface is obviously liquid in behaviour, i.e. is readily distorted and reconstitutes itself when broken without leaving frayed edges. Whether a pellicle is present or not, a determination of its tension and of its behaviour on stretching will give valuable information on its character, and on the order of magnitude of surface forces to be reckoned with in the living cell.

It is obvious that the classical methods of determining surface tension cannot be used with cells, but several independent methods available show that (1) the tension, i.e. the sum of surface and elastic tensions (due to a pellicle) is low, less than 1 dyne/cm.; (2) the surface has elastic properties. These methods are outlined below. It must be borne in mind that all measurement of tension involving deformation of whole cells cannot distinguish between elastic forces at the surface and those of the interior. If the interior is gelled, surface tension equations cannot apply. If the interior is an elastic liquid (see Seifritz, 1929) its effect would be added to that of the surface. In view of the well-known elasticity of films, especially of protein monolayers, it seems reasonable to attribute elastic properties to the surface.

Historically the actual measurement of tension at the cell surface is recent, the assumption having been made that its value was about that between oil and aqueous solutions, around 20 dynes/cm. Pfeiffer (1890) studied what he called the cohesive force of protoplasm. He determined the weight attached to the protoplasmic thread of *Chondrioderma*, a slime-mould (in air), which did not stretch it. A filament 0.3 mm. in diameter would just support 3.5 mg. If we assume that this is all due to the tension around the circumference of the thread, the value is 35 dynes/cm., a quite reasonable figure. One would like to know the tension when the filament is immersed in water. Czapek's (1911) belief that the tension of a cell surface is the same as that of a solution whose surface tension is 68% of that between water and air is based on

comparable with the yield data from other years and other stations. Moisture sampling therefore would not have been eliminated but the number of samples required would be greatly reduced.

The summary of the several-crop tests clearly indicates that where large differences in dry matter percentage occur between forage crop species, that are included in any one test, it is necessary to take moisture samples by some method that will bring out these differences.

SAMPLING STUDY

Test of Various Grass Species

A summary of the dry matter percentage data and the variance analysis of these data are presented in Tables 3 and 3a, respectively.

TABLE 3.—DRY MATTER PERCENTAGES, AVERAGE OF SIX REPLICATES, AS DETERMINED FROM DIFFERENT SIZES OF MOISTURE SAMPLES FOR A TEST OF SEVEN GRASS SPECIES

Grass species	Size of sample and sample number							Means of species
	0.5 pound			0.75 pound		1.5 pounds		
	1a	1b	1c	2a	2b	3a	3b	
<i>Agropyron glaucum</i>	51.6	50.5	50.1	49.4	49.9	50.8	50.6	50.4
<i>Elymus virginicus</i>	49.8	49.9	49.9	49.1	49.5	49.8	49.4	49.3
<i>Agropyron desertorum</i>	50.6	49.4	49.3	48.4	48.0	46.6	45.4	48.3
<i>Elymus canadensis</i>	47.3	46.9	47.4	46.4	47.6	47.3	47.1	47.1
<i>Agropyron cristatum</i>	48.2	47.4	47.6	46.7	46.4	44.3	45.4	46.6
<i>Agropyron elongatum</i>	43.1	41.6	43.9	41.8	42.5	42.0	42.2	42.4
<i>Bromus inermis</i>	36.0	35.3	34.9	33.6	33.4	31.8	32.0	33.9
Sample means	46.6	45.8	46.2	45.1	45.0	44.7	44.6	
Means of sample size	46.2			45.0		44.6		

Least significant difference between means of species, 1 per cent level = 2.4 per cent.

Least significant difference between means of sample size, 5 per cent level = 0.5 per cent.

TABLE 3a.—VARIANCE ANALYSIS OF THE DRY MATTER DATA OF THE TEST OF SEVEN GRASS SPECIES

Source of variation	Degrees of freedom	Sum of squares	Mean square	F. value
Species	6	8192.72	1365.45	86.59**
Replicates	5	107.76	21.55	1.37
Error (a)	30	473.09	15.77	
Plots	41	8773.57		
Between sample size	2	146.54	73.28	49.51**
Within sample size	4	16.20	4.05	2.74*
Species × between sample size	12	142.08	11.84	8.00**
Species × within sample size	24	45.79	1.91	1.29
Error (b)	210	311.35	1.48	
Total	293			

* Significant at the 5 per cent level.

** Significant at the 1 per cent level.

Table 3a shows that the grass species differ very significantly in dry matter content. It is seen in Table 3 that *Bromus inermis* produced the lowest average per cent dry matter, 33.9 per cent, and *Agropyron glaucum* produced the highest percentage with 50.4 per cent. In this test the replicates were not significantly different for dry matter percentage. The differences between the means of the different sizes of samples were highly significant. The means of the 0.75-pound samples and the 1.5-pound samples are very significantly lower than the mean of the 0.5-pound samples, even though these differences were extremely small. The general indication from these data is that as the size of the moisture sample decreased the percentage of dry matter increased. One plausible explanation of this peculiarity might be that the scale used for weighing the green samples may have had a small constant error, although the scale was thoroughly checked before each experiment. If this error was such that instead of obtaining the required weight of 1.5, 0.75, or 0.5 pounds of green material, the weight required plus an additional constant amount was obtained, then as the size of sample decreased the constant error would become proportionately larger. The extra amount of green material would thus cause the smaller samples to show a slightly higher dry matter percentage.

The variation within each size of sample was also greater in the 0.5-pound samples than in the larger samples. The only significant difference between samples within a size is between samples 1a and 1b. This difference is very small but it does tend to indicate that the 0.5-pound samples are not as reliable as the 1.5-pound samples. The significant interaction of species x between sample size is a further indication that the small samples are not as reliable as the larger samples. This significant interaction might possibly have been caused by small samples giving a lower percentage dry matter than the larger samples in some of the more leafy species. The interaction of species x within sample size is not significant. This would indicate that each size of sample was consistent in sampling any particular species.

The very low variability of this sampling test tends to exaggerate very small differences and make them seem important. In actual forage crop testing the variability, due to uncontrollable factors, is so much larger that these very small differences no longer seem important. The coefficient of variability for the sampling data is only 2.68 per cent, whereas on the actual yield data the coefficient of variability for this test is 13.78 per cent.

(a) Number of Samples Per Plot

The standard method of moisture sampling used at the Dominion Forage Crops Laboratory, Saskatoon, consists of taking one 1.5-pound sample from each plot in a test. It was desirable to ascertain the relative efficiency of this one sample per plot as compared to two or more samples per plot. Using a procedure given by Snedecor (5) and used by Torrie *et al.* (6), it is possible to estimate the relative efficiency of increasing both the number of samples per plot and the number of plot replications.

Torrie *et al.* pointed out that field experiments in which samples are taken to represent the whole plot have two sources of random variation, the sampling and experimental errors. The sampling error is the variance

between samples within a plot. Experimental error is made up of two sources of variation, the random variation of plots within a replicate and the sampling error. The random variation of plots within a replicate is designated as A and the variance of the mean of k samples about the mean of the plot is B/k . Thus the experimental error variance in terms of plot means is $A + \frac{B}{k}$, which in terms of individual samples would be $k\left(A + \frac{B}{k}\right) = kA + B$. The estimated variance of a species mean on a single plot basis, \bar{V}_x , would be $\bar{V}_x = \frac{kA + B}{kr}$, where k is the number of samples and r is the number of replicates.

The dry matter percentage data, as determined from each sample, were grouped according to size and a separate analysis was made of each group. The summary of these analyses appears in Table 4.

The experimental error, error (a) Table 4, is represented by $kA + B$ which, in the case of 1.5-pound samples, is equal to 5.33. The sampling error, error (b) is represented by B and is equal to 0.83. The number of samples per plot, K , equals 2. Thus the value of A is 2.25. From these data \bar{V}_x can now be calculated.

$$\bar{V}_x \text{ where } k = 2, r = 6, = \frac{kA + B}{kr} = \frac{2(2.25) + 0.83}{2 \times 6} = 0.444$$

$$\bar{V}_x \text{ where } k = 1, r = 6, = \frac{1(2.25) + 0.83}{1 \times 6} = 0.513$$

$$\text{the relative precision factor in per cent} = \frac{0.513}{0.444} \times 100 = 116$$

TABLE 4.—SUMMARY OF THE VARIANCE ANALYSES OF EACH OF THE SAMPLE SIZES, FOR THE TEST OF SEVEN GRASS SPECIES

Variation due to	0.5-Pound samples		0.75-Pound samples		1.5-Pound samples	
	DF	Mean square	DF	Mean square	DF	Mean square
Species	6	532.93	6	378.75	6	477.45
Replicates	5	13.04	5	7.87	5	4.79
Error (a)	30	7.77	30	6.17	30	5.33
Main plots	41	—	41	—	41	—
Samples	2	7.03	1	0.006	1	0.15
Error (b)	82	1.79	41	1.27	41	0.83
Total	125	—	83	—	83	—

It is thus seen that two samples per plot increased the estimated sampling efficiency by 16 per cent. The coefficient of variability for sampling in the analysis shown in Table 4 for the 1.5-pound samples was 2.04 per cent. The dry yield data, as determined from using moisture sample 3b, showed a coefficient of variability of 13.02 per cent. It is quite

obvious that an increase of 16 per cent in the moisture sampling would affect the yield data very little. Thus, for practical purposes, one 1.5-pound moisture sample per plot would be essentially as good as two samples per plot.

The above procedure was also used to determine the relative efficiency of the different sizes of samples. The estimated variances of species means on a single plot basis, V^- values, and the relative precision factors, using different numbers of samples, were calculated for the three sizes of moisture samples, and are shown in Table 5.

TABLE 5.—THE ESTIMATED VARIANCES OF SPECIES MEANS AND RELATIVE PRECISION FACTORS FOR DIFFERENT NUMBERS OF SAMPLES PER PLOT WITH SIX REPLICATES, FOR DIFFERENT SIZES OF MOISTURE SAMPLES

Sample size (pounds)	Mean square		Estimated variance of a plot A	Estimated variance of a species mean and relative precision factor					
	Experimental Error kA + B	Sampling Error B		k = 1		k = 2		k = 3	
				\bar{V}_x	P.F.*	\bar{V}_x	P.F.	V_x	P.F.
0.5	7.77	1.79	1.99	0.630	70	0.481	92	0.432	103
0.75	6.17	1.27	2.45	0.620	72	0.514	86	0.479	93
1.5	5.33	0.83	2.25	0.513	87	0.444	100	0.421	105

* P.F. = Precision factor or estimated efficiency in per cent.

The relative precision factors in Table 5 are expressed in terms of $k = 2, r = 6$, for 1.5-pound samples, as 100. It is seen that one 1.5-pound moisture sample per plot is a relatively more efficient sampling procedure than using one 0.5-pound sample or one 0.75-pound sample per plot. It is also noted that one 1.5-pound sample per plot gives approximately the same relative efficiency as two 0.75-pound samples per plot and only slightly less than two 0.5-pound samples per plot.

On the basis of the above data it is apparent that the use of one 1.5-pound sample per plot, as a standard method of sampling this six replicate test, is justified. The data further indicate that the moisture samples that were less than 1.5 pounds were more variable and hence not as reliable as the larger samples.

(b) Sampling Methods

It would be advantageous to have sampling methods that were shorter and less detailed, than the standard method of sampling, but which would give results that were essentially as accurate. For tests involving 6 replicates some suggested shorter methods are:

1. One 0.5-pound sample of green material taken from each plot. The samples from the first three replicates would be bulked for each species, and dried as one 1.5-pound sample. Similarly the three samples for each species for the last three replicates would be bulked and dried as another sample. The dry matter percentage as determined for each sample would then be used to calculate the dry yield for each particular species in its respective replicates. By using this method only one-third of the normal number of samples would be dried.

2. One 0.75-pound sample taken from each plot. The two samples, for each species, from replicates 1 and 2, would be bulked and dried as one sample. Similarly the samples from replicates 3 and 4, and 5 and 6 would be bulked and dried. The dry matter percentage would then be used to calculate the dry yield of the particular species in its respective replicates. This method would reduce the number of samples to be dried to one-half of the usual number.

3. One 1.5-pound sample taken from each plot in one replicate, chosen at random. The dry matter percentage for each species from this one replicate would then be applied to all replicates. This would reduce the number of samples to one-sixth of the normal number required.

4. One 1.5-pound sample from each plot in each of two replicates, chosen at random. The two determinations for each species would then be averaged and applied to all replicates. In this case only one-third of the normal number of samples would be required.

In order to obtain a comparison of these methods with the standard method the available data were arranged to conform, as closely as possible, to the suggested methods. The first two methods could not be made up as described because when carrying out the sampling study each sample was dried individually. Therefore, Method 1, as it appears in Table 6, was made up from a random selection of one 0.5-pound sample from each plot and then the average percentage dry matter determined for the first three replicates and for the last three replicates in each species. Similarly for Method 2 individual determinations were selected from each plot, at random, and averaged for each set of two replicates. In Methods 3 and 4 the dry yield data were determined from the dry matter percentages of sample 3b. Sample 3a was used as the check method; this also was chosen at random.

TABLE 6.—SUMMARY OF THE DRY YIELD DATA OF THE TEST OF SEVEN GRASS SPECIES, AS DETERMINED BY THE DIFFERENT SAMPLING METHODS, WITH THE F VALUES AND LEAST SIGNIFICANT DIFFERENCES INCLUDED

Grass species	Key to species	Average yield in pounds per plot METHODS				
		1	2	3	4	Check
<i>Agropyron elongatum</i>	A	6.23	6.20	6.06	6.26	6.15
<i>Agropyron glaucum</i>	B	5.99	5.99	5.78	6.01	6.07
<i>Elymus canadensis</i>	C	5.00	4.99	4.56	5.03	4.97
<i>Agropyron desertorum</i>	D	4.61	4.44	4.15	4.10	4.25
<i>Agropyron cristatum</i>	E	3.79	3.72	3.67	3.59	3.51
<i>Bromus inermis</i>	F	3.73	3.58	3.69	3.31	3.41
<i>Elymus virginicus</i>	G	2.73	2.66	2.63	2.75	2.74
F values		27.76**	33.16**	29.52**	34.18**	28.74**
Least significant difference 1 per cent point		0.94	0.88	0.87	0.90	0.97

** Significant at the 1 per cent level.

For each method the dry yield per plot was determined and a separate variance analysis was conducted. Table 6 presents the average dry yield for each species, as determined by the various methods, and also includes the F values and the least significant differences as determined by the variance analyses.

The species in Table 6 have been arranged in order of highest yield according to the check method of sampling, the highest yielding species being designated with the letter A. It is seen that the order of the species for Methods 1, 2, and 4 is the same as given by the standard or check method of sampling. In Method 3 a slight change occurred which resulted in species F yielding slightly more than species E. The difference in yield between these two species is not significant. Throughout the whole table there is a great deal of similarity between all sets of yield data. The F values are all at much the same level of significance. Some differences do occur, however, in the significant relationships between species. For example, the check method shows no significant difference existing between the species E and G, but Methods 1, 2, and 3 show that the difference in yield between these two species is highly significant.

In order to further compare the different methods with the check method of sampling, the dry yield data, as determined by each sampling method, were analysed in conjunction with the dry yields of the check method using the split-plot type of variance analysis for each comparison. The summary of these variance analyses is presented in Table 7.

Table 7 shows that Methods 1, 2, and 3 each gives a significantly different average yield than that given by the check method of sampling. It is also seen that when each of these methods was compared to the check

TABLE 7.—SUMMARY OF THE VARIANCE ANALYSES OF THE DRY YIELD DATA, OF THE GRASS SPECIES TEST, AS DETERMINED BY EACH SAMPLING METHOD, IN DIRECT COMPARISON WITH THE DRY YIELD DATA DETERMINED BY THE CHECK METHOD OF SAMPLING

Variation due to	Degrees of freedom	Mean squares			
		Sampling methods in comparison with check			
		1	2	3	4
Species	6	20.3344**	20.9094**	19.4833**	21.8249**
Replicates	5	6.9456**	6.7487**	6.7463**	6.8100**
Error (a)	30	0.7042	0.6669	0.6589	0.6807
Plots	41	—	—	—	—
Methods	1	0.4060**	0.1009*	0.1360**	0.0009
Methods × species	6	0.0947**	0.0462*	0.1702**	0.0280
Error (b)	35	0.0183	0.0144	0.0161	0.0177
Total	83	—	—	—	—

* Significant at the 5 per cent point.

** Significant at the 1 per cent point.

method the interaction of methods X species was significant. This indicates that the relationships between the species, as determined by the check method of sampling, were significantly changed when sampling Methods 1, 2 and 3 were used. It can therefore be stated that, for this particular test, sampling Methods 1, 2, and 3 were unreliable. When Method 4 was compared to the check method it was found that no significant difference existed between the two methods nor was there a significant interaction. This is a very good indication that Method 4 was as reliable as the check method of sampling for this particular comparative test.

Sweet Clover Variety Test

The second comparative test used in the sampling study was a sweet clover variety test. The summary of the dry matter percentages, as determined from the different sizes of samples for each variety, appears in Table 8.

In this test the plot of the Improved Alpha variety in the sixth replicate was missing. The method of analysis used was that set forth by Anderson (1) whereby the whole-plot treatments, varieties in this instance, were analysed by estimating the missing whole-plot, and the sub-plot treatments, that of samples, were analysed by the method of proportionate sub-class numbers, in which only the existing plots were considered. Table 8a shows the variance analyses of both the whole-plot and sub-plot treatments.

It is seen that the varieties differed very significantly in the amount of dry matter that they contained, with Erector giving the highest average percentage, 40.9 per cent, and Arctic the lowest average percentage dry matter, 29.0 per cent. Erector was significantly higher than all other varieties and Aura was significantly higher than the remaining varieties. The analysis of variance also showed the replicates to be significantly different.

TABLE 8.—DRY MATTER PERCENTAGES, AVERAGE OF SIX REPLICATES, AS DETERMINED BY DIFFERENT SIZES OF MOISTURE SAMPLES FOR SIX SWEET CLOVER VARIETIES

Varieties	Size of sample and sample number							Varietal means
	0.5-pound			0.75-pound		1.5-pound		
	1a	1b	1c	2a	2b	3a	3b	
Erector	40.2	40.5	40.4	41.2	40.5	41.3	42.0	40.9
Aura	35.7	36.6	35.4	36.1	36.0	36.8	37.1	36.2
Redfield Yellow	31.0	31.3	31.8	30.0	30.6	30.6	31.5	31.0
Improved Alpha*	30.6	30.0	30.0	28.8	29.7	29.7	30.2	29.9
<i>M. alba</i> S-567	30.6	28.9	29.3	28.9	30.0	30.4	30.3	29.8
Arctic	29.2	28.2	29.0	29.1	28.6	29.4	29.3	29.0
Sample means	32.9	32.7	32.7	32.5	32.7	33.1	33.5	
Means of sample size	32.8			32.6		33.3		

* Averages of five replicates.

Least significant difference between varietal means at the 1 per cent level = 3.4 per cent.

Least significant difference between means of sample sizes at the 5 per cent level = 0.5 per cent.

TABLE 8a.—VARIANCE ANALYSIS OF THE DRY MATTER PERCENTAGES FROM THE SWEET CLOVER VARIETY TEST

Whole-plot treatments				
Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Replicates	5	614.87	122.97	4.08**
Varieties	5	4749.57	949.91	31.51
Error (a)	24	723.63	30.15	
Total	34	6088.07		

Sub-plot treatments				
Whole plots	34	6024.73		
Between sample sizes	2	20.70	10.35	4.14*
Within sample sizes	4	4.27	1.07	
Strains × between samples	10	20.80	2.08	
Strains × within samples	20	29.58	1.48	
Error (b)	174	434.20	2.50	
Total	244	6534.28		

* Significant at the 5 per cent level.

** Significant at the 1 per cent level.

A significant difference between sample sizes was found to exist. The 1.5-pound samples showed a significantly higher dry matter percentage than both the 0.75 and the 0.5-pound samples. In this test there appears to be no relationship between size of sample and dry matter percentage such as was found in the previous test of grass species. Neither of the interactions was found to be significant. Therefore the sampling of the different varieties was consistent for each size of sample.

Using the procedure previously described the estimated variances of varietal means on a single plot basis and the relative precision factors were determined for each size of sample with $r = 6$ and $k = 1$ to 3. These data have been summarized and presented in Table 9.

TABLE 9.—THE ESTIMATED VARIANCES OF VARIETAL MEANS AND RELATIVE PRECISION FACTORS FOR DIFFERENT NUMBERS OF SAMPLES PER PLOT WITH SIX REPLICATES, FOR DIFFERENT SIZES OF MOISTURE SAMPLES

Sample size (pounds)	Mean square		Estimated variance of a plot A	Estimated variance of a varietal mean and the relative-precision factor					
	Experimental Error kA + B	Sampling Error B		k = 1		k = 2		k = 3	
				\bar{V}_x	P.F.*	\bar{V}_x	P.F.	\bar{V}_x	P.F.
0.5	20.395	2.755	5.880	1.439	42	1.210	50	1.133	54
0.75	7.456	2.523	2.467	0.832	73	0.621	98	0.551	110
1.5	7.289	1.325	2.982	0.718	85	0.607	100	0.571	106

* P.F. = Precision factor or estimated efficiency in per cent.

The relative precision factors in Table 9 are expressed in terms of $k = 2$, $r = 6$, for 1.5-pound samples, as 100. It is seen that one 1.5-pound sample per plot in this test is a more efficient method of sampling than three 0.5-pound samples per plot, and also more efficient than one 0.75-pound sample per plot. Compared to two 1.5-pound samples per plot one 1.5-pound sample per plot is 85 per cent as efficient. It is therefore seen that the standard method of sampling has been justified in view of the relatively small increase in efficiency gained from double the amount of moisture sampling.

Again, in order to evaluate possible shorter sampling methods, the same methods as described for the grass species test were applied to the sweet clover yield data. The summary of the average yield data for each variety, as determined by each method, and the F values from the individual variance analyses appear in Table 10. The varieties are arranged in order of highest yield as determined by the check method of sampling.

TABLE 10.—SUMMARY OF THE DRY YIELD DATA OF THE SWEET CLOVER VARIETY TEST, AS DETERMINED BY THE DIFFERENT SAMPLING METHODS, WITH THE F VALUES AND LEAST SIGNIFICANT DIFFERENCES FROM THE VARIANCE ANALYSES INCLUDED

Varieties	Key to varieties	Average yield in pounds per plot				
		METHODS				
		1	2	3	4	Check
Redfield Yellow	A	5.61	5.27	5.12	5.31	5.42
Erector	B	4.90	4.99	4.43	5.14	5.02
Arctic	C	4.30	4.38	4.35	4.40	4.33
Aura	D	3.47	3.25	3.43	3.65	3.41
Improved Alpha	E	3.40	3.33	3.11	3.34	3.32
<i>M. alba</i> S-567	F	3.03	3.12	3.01	3.14	3.16
F. value		4.72**	5.06**	4.10**	4.37**	5.22**
L.S.D. (1)†		1.82	1.62	1.66	1.76	1.67
L.S.D. (2)†		1.93	1.72	1.76	1.86	1.76

** Significant at the 1 per cent level.

† Least significant difference for comparisons of all varieties except Improved Alpha.

‡ Least significant difference for comparison of Improved Alpha with any other variety.

The ranking of the varieties, Table 10, for sampling Methods 1, 3, and 4 are the same as for the check method. Method 2 shows a small change in the order of the varieties D and E. According to the check method variety A is significantly higher than varieties D, E, and F, and variety B is significantly higher than varieties E and F. Method 1 shows the same significant differences except that variety B in this case is not significantly different to variety E. Method 2 shows variety A with the same significance but variety B in this case is significantly different to varieties D, E, and F. By Method 3, variety A is significantly different to varieties D, E, and F but variety B is not significantly different to any other variety. The fourth method of sampling shows varieties A and B significantly different to varieties E and F but not to D.

In order to find if the above changes were significant the dry yield data determined by each sampling method were separately compared to those of the standard method of sampling using the split-plot type of variance analysis. The summary of these variance analyses appears in Table 11.

Table 11 shows that Method 3 is the only method of sampling that differs significantly from the check method. Since the interaction of methods \times varieties is not significant when Methods 1, 2, and 4 are each compared to the check method, it can therefore be concluded that the changes in ranking and in the relationships of the varieties, as previously outlined, are not significant. It can thus be stated that for this sweet clover variety test the moisture sampling Methods 1, 2, and 4, were just as reliable as the standard method of sampling.

TABLE 11.—SUMMARY OF THE VARIANCE ANALYSES OF THE DRY YIELD DATA OF THE SWEET CLOVER VARIETY TEST, AS DETERMINED BY EACH SAMPLING METHOD, IN DIRECT COMPARISON WITH THE DRY YIELD DATA DETERMINED BY THE CHECK METHOD OF SAMPLING

Whole-plot treatments					
Source of variation	Degrees of freedom	Mean squares			
		Sampling methods in comparison with check			
		1	2	3	4
Replicates	5	32.9451	32.3515	30.3459	32.4588
Varieties	5	11.4756	10.6288	9.7268	10.6768
Error (a)	24	2.2688	2.0237	2.0883	2.1950
Total	34				
Sub-plot treatments					
Whole-plots	34				
Methods	1	0.0013	0.0148	0.6762**	0.0588
Methods \times varieties	6	0.0388	0.0119	0.1302*	0.0699
Error (b)	28	0.0972	0.0801	0.0456	0.0812
Total	69				

* Significant at the 5 per cent level.

** Significant at the 1 per cent level.

Brome Grass Strain Test

The dry matter percentage data of the brome grass strain test are summarized in Tables 12 and 12a. It is seen that the differences in dry matter percentages between strains are highly significant even though the maximum difference between any two means is only 3.8 per cent. There is also a significant difference between the percentages of dry matter determined from the different sizes of samples. The necessary difference between size of sample means is 0.6 per cent; thus the 0.5-pound samples gave a significantly higher dry matter percentages than did the 1.5-pound

samples, the difference between them being 0.7 per cent. Although this difference is shown to be significant it can hardly be called an important difference and a rigid distinction between sample size on the basis of this seems hardly justifiable. However, the results found here are in agreement with those of the test of various grass species, that is, there is a definite trend for dry matter percentage to be inversely proportional to the size of the sample used for the determination.

TABLE 12.—DRY MATTER PERCENTAGES, AVERAGE OF FOUR REPLICATES, AS DETERMINED FROM DIFFERENT SIZES OF MOISTURE SAMPLES FOR SIXTEEN STRAINS OF BROME GRASS

Strains	Size of sample and sample number						Means of strains
	0.5 pound			0.75 pound		1.5 pounds	
	1a	1b	1c	2a	2b	3	
S-1264	39.2	40.8	38.9	40.2	39.6	40.5	39.9
S-1224	40.4	39.8	40.6	38.8	38.8	38.7	39.6
S-1258	39.8	39.2	39.9	39.4	38.7	39.3	39.4
S-1229	40.2	39.0	39.8	39.4	39.2	38.3	39.3
S-1262	40.6	38.3	38.7	38.6	38.3	37.6	38.7
S-1260	39.4	38.5	37.9	39.4	38.3	38.0	38.6
S-1256	37.9	38.9	38.7	38.4	38.6	37.8	38.4
S-1265	38.1	37.1	42.4	37.2	37.2	37.2	38.2
S-1263	38.5	38.3	37.5	38.6	37.8	38.1	38.1
S-1261	37.5	38.6	38.3	37.4	37.5	37.0	37.7
S-1259	37.5	38.8	38.1	37.7	36.8	37.0	37.7
S-1227	36.5	35.8	37.3	37.6	38.4	37.6	37.2
S-1257	37.7	38.3	36.1	37.5	36.4	36.4	37.1
S-1255	36.2	36.0	36.9	37.2	36.4	35.8	36.4
Commercial	36.5	37.1	36.9	36.1	35.3	36.0	36.3
Superior	35.8	36.5	35.8	36.4	36.8	35.6	36.1
Sample means	38.2	38.2	38.4	38.1	37.8	37.6	
Means of sample size	38.3			37.9		37.6	

Least significant difference between means of strains, 1 per cent level = 2.3 per cent.

Least significant difference between means of sample size, 1 per cent level = 0.6 per cent.

TABLE 12a.—VARIANCE ANALYSIS OF THE DRY MATTER PERCENTAGES OF THE BROME GRASS STRAIN TEST

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Strains	15	510.19	34.0127	3.82**
Replicates	3	118.16	39.3867	4.42**
Error (a)	45	401.01	8.9113	
Plots	63	1029.36		
Between size of samples	2	25.92	12.9600	5.85**
Within size of samples	3	5.00	1.6667	
Between sample × strains	30	70.53	2.3510	1.06
Within sample × strains	45	132.77	2.9504	1.33
Error (b)	240	532.13	2.2172	
Total	383	1795.71		

** Significant at the 1 per cent level.

Precision factors were determined for the 0.5 and 0.75-pound samples and are presented in Table 13. In this test only one 1.5-pound sample could be taken so that it was not possible to estimate the variance of the strain means for different numbers of 1.5-pound samples.

TABLE 13.—THE ESTIMATED VARIANCES OF STRAIN MEANS AND RELATIVE PRECISION FACTORS FOR DIFFERENT NUMBERS OF SAMPLES PER PLOT WITH 4 REPLICATES, FOR TWO SIZES OF MOISTURE SAMPLES

Sample size (pounds)	Mean square		Estimated variance of a plot A	Estimated variance of a strain mean and relative precision factor					
	Experimental Error kA + B	Sampling Error B		k = 1		k = 2		k = 3	
				$V_{\bar{x}}$	P.F.*	$V_{\bar{x}}$	P.F.	$V_{\bar{x}}$	P.F.
0.5	8.33	3.13	1.73	1.22	40	0.82	60	0.69	71
0.75	3.94	1.18	1.38	0.64	77	0.49	100	0.44	111

* P.F. = Precision factor or efficiency in per cent.

The relative precision factors in Table 13 are expressed in terms of $k = 2$, $r = 4$, for 0.75-pound samples, as 100. It is seen that one 0.75-pound sample per plot is more efficient than three 0.5-pound samples per plot, thus further indicating that the smaller sizes of moisture samples are more variable and hence less reliable than the larger moisture samples.

The shorter methods of sampling as used in the two previous tests were also applied to the yield data from the brome grass strain test. In this test, however, there were only four replicates; thus some changes in the sampling methods were necessary. In Method 1 the random 0.5-pound samples from each plot were averaged for the first two replicates for each strain and similarly for the second two replicates. Thus this method in actual use would mean drying two 1-pound samples for each strain instead of two 1.5-pound samples. Methods 2, 3, and 4 were the same as previously described. A further method, number 5, was added to this test. This consisted of taking six random 1.5-pound samples for the whole test, averaging them, and determining the dry yield of each plot on the basis of the average dry matter percentage. The results of the variance analysis of the yield data on the basis of this method of sampling would be essentially the same as they would be if green yields had been used.

The summary of the yield data, as determined by the different methods of sampling, and the F values from the variance analyses of the dry yield data for each method, are presented in Table 14. The strains are listed in order of highest yield as determined by the check method of sampling.

It is seen that there are no significant differences between strains for check method or for Methods 2 and 3, although in all cases the 5 per cent level of significance is approached. Sampling methods 1, 4 and 5 all show the strains to be significant to the 5 per cent point. Several changes occur in the ranking of the strains for yield.

The dry yield data from each sampling method were compared to the dry yields from the standard method of sampling using the split-plot type of variance analysis. The summary of these analyses is presented in Table 15.

TABLE 14.—SUMMARY OF THE DRY YIELD DATA OF THE BROME GRASS STRAIN TEST, AS DETERMINED BY THE DIFFERENT SAMPLING METHODS, WITH THE F VALUES AND LEAST SIGNIFICANT DIFFERENCES FROM THE VARIANCE ANALYSES INCLUDED

Strains	Methods of sampling					
	1	2	3	4	5	Check
S-1265	3.60	3.61	3.22	3.85	3.71	3.66
S-1263	3.56	3.62	3.69	3.55	3.55	3.60
S-1264	3.29	3.24	3.26	3.56	3.19	3.43
S-1258	3.39	3.36	3.19	3.40	3.24	3.38
Superior	3.30	3.43	3.26	3.41	3.54	3.32
Commercial	3.38	3.29	3.50	3.22	3.42	3.27
S-1256	3.26	3.30	3.15	3.22	3.21	3.23
S-1255	3.30	3.30	3.26	3.27	3.40	3.23
S-1260	3.06	3.06	3.20	3.06	3.04	3.07
S-1259	3.15	3.05	2.98	3.15	3.11	3.05
S-1229	3.05	3.14	3.02	3.11	3.02	3.05
S-1257	3.03	3.04	2.97	3.03	3.06	2.95
S-1261	2.96	2.94	2.81	2.97	2.93	2.87
S-1224	2.63	2.56	2.50	2.60	2.50	2.55
S-1262	2.42	2.40	2.38	2.37	2.38	2.36
S-1227	2.19	2.30	2.26	2.27	2.25	2.25
F value	1.98*	1.76	1.88	2.14*	2.13*	1.68
L.S.D.	0.80	—	—	0.83	0.82	—

* Significant at the 5 per cent level.

Table 15 shows that by using sampling methods 3 and 4 a significantly different average yield is obtained. A slightly different average yield is not a very important factor, particularly when the difference is small. Methods 3 and 5 bring about a significant interaction between methods \times strains. This indicates that the yield relationships between the various strains are changed significantly from that of the check method. Therefore

TABLE 15.—SUMMARY OF THE VARIANCE ANALYSES OF THE DRY YIELD DATA, OF THE BROME GRASS STRAIN TEST, AS DETERMINED BY EACH SAMPLING METHOD, IN DIRECT COMPARISON WITH THE DRY YIELD DATA DETERMINED BY THE CHECK METHOD OF SAMPLING

Variation due to	Degrees of freedom	Mean squares				
		Sampling methods in comparison with check				
		1	2	3	4	5
Strains	15	1.2787	1.2719	1.2322	1.3834	1.3490
Replicates	3	3.9695	3.9225**	4.0462**	4.1454**	4.1076**
Error (a)	45	0.7085	0.7440	0.7202	0.7340	0.7270
Plots	63					
Methods	1	0.0118	0.0218	0.0473*	0.0775**	0.0093
Methods \times strains	15	0.0097	0.0109	0.0456**	0.0087	0.0264**
Error (b)	48	0.0087	0.0076	0.0095	0.0095	0.0099
Total	127					

* Significant at the 5 per cent point.

** Significant at the 1 per cent point.

Methods 3 and 5 are not sufficiently reliable for this test. Methods 1, 2, and 4 did not produce a significant interaction; therefore any differences found in the relationships between strains when comparing these sampling methods to the check method could be accounted for on the basis of chance variations. It can therefore be stated that for this test the shorter sampling methods 1, 2, and 4, have been as reliable as the standard method of sampling.

DISCUSSION

No data regarding the correlation of dry weights with green weights have been included in this study. Since the green yield of any plot consists of the dry yield plus a variable amount of water, and since the dry yield depends on the green yield and the weight of the water, a correlation between any two of these factors would not be justified. With such a relationship between the variables the correlation coefficient would necessarily be high. If there was a perfect correlation between green and dry yields it could be concluded that green yields would be as reliable as dry weights. However, once the correlation coefficient was less than 1 the reliability of the green yields would be doubtful. This can be illustrated by the following example. Test number 10, Table 5, was a sweet clover variety test, the green yields of which were shown as giving quite different results to that of the dry yields. When the green yields in this test were correlated with the dry yields the coefficient of correlation was found to be $+0.98$.

The short sampling methods that were suggested in this paper should be considered from the practical viewpoint. Methods 1 and 2, involving the combination of small amounts of green material, might, in actual practice, be found to be somewhat complicated and subject to considerable error. One such possible error might be the combination of samples from plots of different species or varieties being sampled. It has also been shown that considerable variation in percentage dry matter occurred when the smaller samples were used. This variation might be due, in part at least, to inaccurate field scales. Therefore these methods, although less time-consuming, might prove to be insufficiently reliable.

Sampling methods 3 and 4 would be very desirable methods since they are relatively easy to employ and would considerably reduce the number of moisture samples. It has been shown, however, that Method 3 in no case gave results that were as reliable as the standard method of sampling. Method 4 on the other hand, in all three tests studied, consistently gave results that were as reliable as the standard method. It thus appears that this latter method of sampling, which consists of sampling two random replicates, using the standard method, and averaging the two dry matter determinations for each variety or treatment, has definite possibilities. It would be highly desirable, however, to test this method again in other years, under different growing conditions, and using different comparative tests.

In dealing with the one-crop tests earlier in the study the conclusion was drawn to the effect that reliable comparisons in the one-crop tests could be made on the basis of green weights, or, for year comparisons, on dry weights based on the average of a few random 1.5-pound moisture

samples. Since the brome grass strain test was a one-crop test it seemed desirable to test that suggested method of sampling. Table 15 shows that Method 5 was not as reliable as the check method of sampling; thus these results tend to contradict the earlier conclusion. However, when it is considered that the test of brome grass strains is an advanced test of many similar strains of one grass species and that very accurate comparisons are necessary in order to bring out the differences, then one should not expect a non-detailed method of sampling to bring out small differences. The one-crop tests discussed earlier were tests of a preliminary nature where fairly large differences between strains could be expected to be found. In such cases this Method 5 should prove to be as satisfactory as more detailed sampling methods.

SUMMARY

1. Significant differences in dry matter percentages were found to exist between strains, varieties, and species of all of the forage crops studied.
2. Significant differences between strains or varieties of a crop did not necessarily justify complete moisture sampling in a comparative test.
3. It was shown that where comparative tests of forage crops for hay yield did not include markedly different varieties or species, then moisture sampling on the basis of one moisture sample per plot was not necessary. Several random samples for the whole test would be sufficient to reduce green yields to dry or hay yields for inter-year comparisons.
4. Where the comparative tests of forage crops for hay yields included more than one species, and where species or varieties differed markedly in dry matter percentage, such as in the case of sweet clover varieties, or where there was an advanced test of many very similar strains, then it was necessary to make moisture determinations on a fairly complete basis.
5. Moisture samples of 0.5 and 0.75 pounds in green weight were found to give significantly different dry matter percentages than the 1.5-pound samples.
6. The smaller moisture samples were shown to be more variable than the larger samples. In one case one 1.5-pound sample per plot was as efficient as three 0.5-pound samples per plot.
7. More than one 1.5-pound sample per plot was found to be unjustified, in the material tested.
8. Moisture sampling on the basis of one 1.5-pound sample per plot in two random replicates and averaging of the two determinations for each variety or treatment was found, in the three tests studied, to be essentially as reliable as sampling every plot in the tests.

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VARIATIONS IN THE ESTRONE CONTENT OF PREGNANT MARES' URINE¹

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INTRODUCTION

The examination of mares' urine samples taken between the fifth and ninth months of pregnancy disclosed wide variations in the concentration of estrone for mares at the same stage of pregnancy. It was decided to determine the urinary estrone concentration for a single mare throughout the entire gestation period and to compare these values with those for the previously-examined mares at identical stages of pregnancy.

Limited literature references to the estrone content of pregnant mares' urine (P.M.U.) disclose wide variations and infer that the values obtained depend on the method applied. Cole and Hart (3), employing a rat test, report a maximum estrone value of 17,500 rat units (17.5 mg.) per litre of urine at 240 days of pregnancy. Cole and Saunders (2) report maximum concentrations of 16,000 and 33,000 rat units at 200 to 275 days. Mayer *et al.* (5) using a chemical technique, in contrast to the above bioassay techniques, found 17.1 mg. of estrone per litre at the fortieth day of pregnancy. Selye (6) reports a concentration of 100 milligrams per litre but the method applied is not stated. The data to be reported are based on a chemical method involving colorimetry.

PROCEDURE

Collection of Urine

Since the mare under study was a working horse, only overnight collections were made. The collection equipment consisted essentially of a heavy curved rubber tube held in position by a light canvas harness. Specimens were obtained at approximately weekly intervals and averaged about one-half gallon in volume. When specimens were lost during the collection period or were low in volume another specimen was secured on the following night. As the collecting equipment did not always permit total retention of urine, no measurements of urine volume were recorded.

Determination of Estrone

The method used for the determination of estrone is based on modifications of the methods of Kober (4), Venning *et al.* (7) and Bachman and Pettit (1). An acid hydrolysis of the urine to obtain the hormone in the free form is followed by several benzol extractions. The combined benzol extracts are washed successively with water, bicarbonate, carbonate, sulphuric acid, and water and then made up to volume. Suitable aliquots, depending on the hormone concentration, are allowed to react with phenol-sulphonic acid reagent in the presence of heat. After cooling, the addition of water, and successive boiling and cooling, a definite amount of sulphuric acid is added to develop the characteristic pink colour. Readings taken

¹ Contribution No. 165 from the Division of Chemistry, Science Service, Department of Agriculture, Ottawa, Canada.

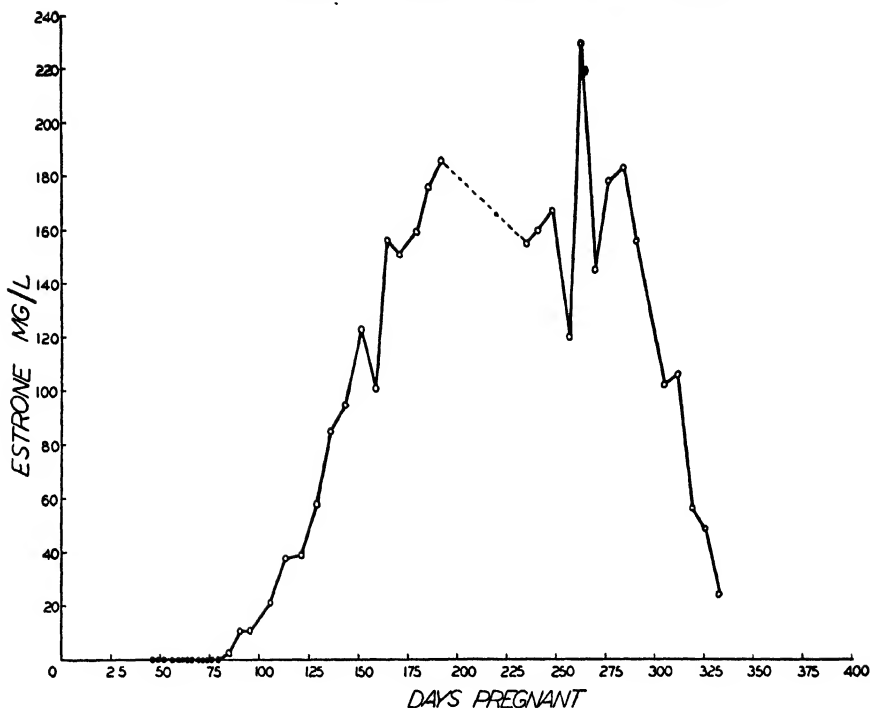


FIGURE 1. The urinary estrone concentration of Mare 10 throughout pregnancy.

NOTE.—Due to technical difficulties data were not obtainable between the 192nd and 235th days.

on the Evelyn colorimeter at 515 and 420m μ permit calculation of estrone values corrected for the brown coloration caused by impurities. For ease in calculation, estrone values of urine samples were determined directly from a nomogram based on international standard estone.

RESULTS

The data obtained from urine specimens for a single mare (Mare 10) for the entire gestation period are shown graphically in Figure 1 expressed as milligrams of estrone per litre.

Frequent specimens were examined early in pregnancy but the first detectable amount of the hormone was noted on the eighty-fifth day. From this point the analyses of weekly specimens revealed a rapid increase in estrone concentration until about the 190th day of pregnancy. During the next hundred days the estrone level, while subject to considerable variation, was maintained at an average value of 170 mg./l. A marked decrease in production occurred during the last forty-eight days of gestation and the low value of 24 mg./l was recorded on the day of parturition.

These variations in estrone concentration for Mare 10 follow the same pattern as that found by Cole and Hart (3) but the values obtained are considerably higher than those of the above workers for the entire gestation period. Mayer *et al.* (5) report an earlier appearance of estrone than was found for Mare 10 and a larger amount at the fortieth day of pregnancy.

The estrone values for Mare 10 are compared in Table 1 with data from other P.M.U. specimens obtained from mares at known stages of pregnancy. In making the comparison it is assumed that no marked change occurs in the hormone production rate over a twenty-four hour period and therefore the time of collection of a specimen would have no appreciable effect on its concentration.

Foaling data for these mares indicate that no abnormal gestations occurred.

TABLE 1.—INDIVIDUAL VARIATIONS IN EQUINE URINARY ESTRONE

Mare No.	Days pregnant	Estrone, mg./l	Estrone mare 10*, mg./l
106	157	89	105
21	158	142	101
78	161	102	119
77	162	63	141
115	164	220	156
19	176	160	158
69	199	114	178
149	202	103	175
35	229	100	155
109	242	51	160
137	271	33	145

* Corresponding days pregnant.

It is evident that great variations exist in the amounts of estrone excreted by different mares at the same stage of pregnancy. For example, the estrone concentrations for the first five mares of the above table vary from 63 to 220 mg./l although the urine specimens were collected within the same week of pregnancy.

While the above data refer to randomly selected specimens it is evident that the maximum production of estrone does not always occur at the same time in different mares. This observation is in agreement with the findings of Cole and Saunders (2) who report maximum concentrations at 158, 207, 220 and 270 days.

SUMMARY

The urinary estrone excretion throughout the entire gestation period of a single mare has been determined by a chemical method. Comparisons have been made between the estrone values for this mare and for other mares at the same stage of pregnancy.

The concentration of urinary estrone for a single pregnant mare rose rapidly from the low value of 2.2 mg./l at the 85th day to an average maximum value of 170 mg./l between the 192nd and 235th days. Thereafter it declined steadily to a value of 24 mg./l on the day of parturition.

Great variations exist in the concentration of estrone excreted by different mares at identical stages of pregnancy.

The maximum production of estrone apparently does not always occur at the same time in different mares.

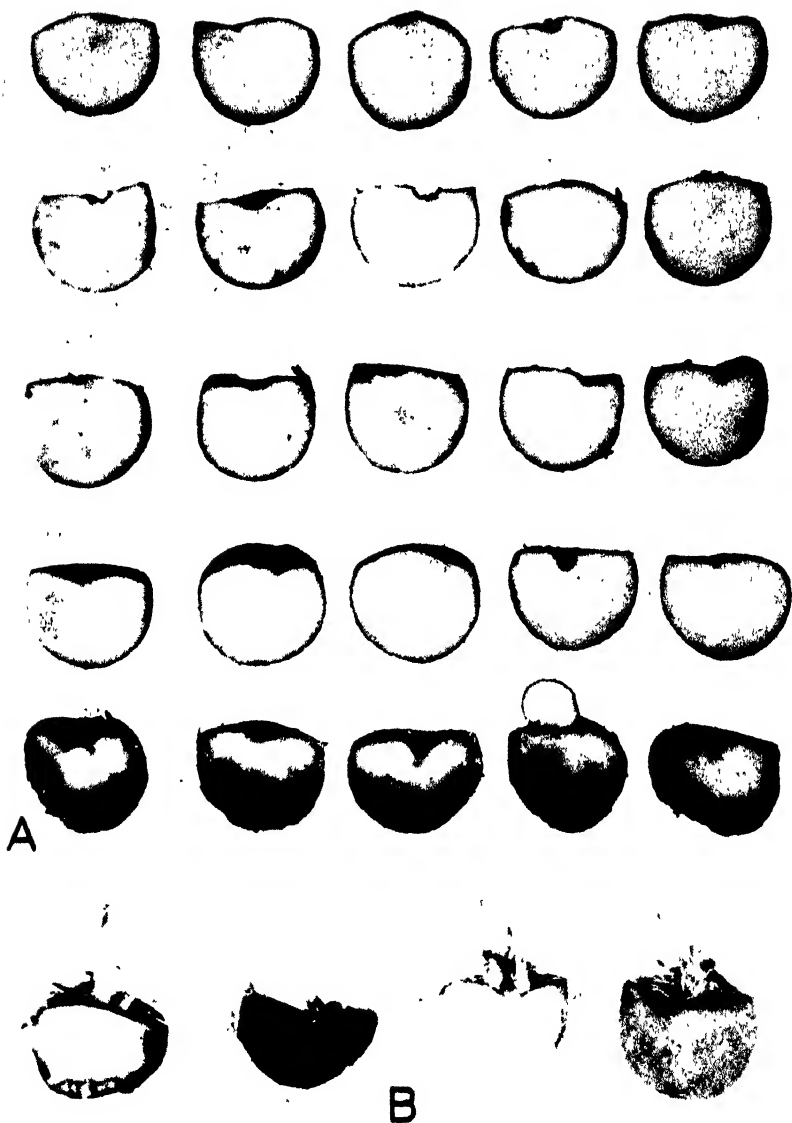


FIGURE 1

- A. Chemical protection of freshly cut potato sets against rotting by *Fusarium coeruleum* in an artificially infested virgin soil. *Top row*, Spergon dust; *second row*, Spergon 1 per cent active chemical in 50 : 50 water-Aerosol solution; *third row*, Fermate dust; *fourth row*, not treated; *bottom row*, uninfested virgin soil, sets not treated.
- B. Contrast in soundness of untreated sets in non-inoculated old cultivated soil (2 halves at left), and in virgin soil (2 halves at right).

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CHLOROTIC BANDING OF CEREAL SEEDLINGS¹

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INTRODUCTION

Chlorotic banding of the leaves of cereal seedlings (Figure 1) is of common occurrence in the spring-wheat region of the Canadian Prairies (3). Hitherto, chlorosis of the banded type has been generally attributed to temperatures at or near the freezing point, frequently with strong winds as an associated factor. Machacek (5), however, reports a case of yellow banding of wheat leaves caused by extreme heat at the soil level. A more severe type of injury in which the tissues are killed, with death of the seedlings often resulting, is frequently encountered. This is usually caused by severe frosts (2). A similar type of injury on late-sown spring oats was attributed by Vanterpool (4) to unseasonable, hot, dry winds one or two days after the plants emerged.

Beginning about May 24, 1948, and for several days thereafter, reports were received of conspicuous yellowing and banding of wheat seedlings over large areas in a general region around Saskatoon and extending northwards and eastwards for about 100 miles. Spring sowing was late. Because of the lack of rain, the surface soil had dried out to seed depth or lower in many fields by the latter part of May. The result was successive periods of seedling emergence in the same field. Unseasonably high air temperatures (Table 2) from May 17 to May 25, and the dry surface soil, strongly indicated that heat rather than cold was the primary contributing factor in last year's yellow banding of wheat seedlings.

Because of the wide distribution of this trouble in north-central Saskatchewan, and the scarcity of published information on this type of heat injury on cereal seedlings, some exploratory work was conducted on the problem in the summer of 1948. The results of the experiments are presented here.

TYPES OF INJURY

Richards (10) has given detailed descriptions of three types of lesions on young cereal plants produced by temperatures at or near the freezing point at the soil surface. The first consists of yellow, chlorotic bands on the first and second leaves, varying from a small speck to bands up to three-fourths of an inch in length (cf. Figure 1). In the second type of injury the banded tissue is bleached and dead, commonly resulting in a characteristic breaking or lopping over of the leaves by subsequent strong winds. The third type of injury consists of a complete collapse of tissue at the soil line. This is the most damaging kind. It is sometimes called the "damping-off" type. Richards has pointed out that the various types are caused by different degrees of intensity of cold at a common point of origin, that is, the soil surface. This is borne out by the author's observations.

¹ Contribution from the Laboratory of Plant Pathology, University of Saskatchewan, Saskatoon, Sask., with financial assistance from the Saskatchewan Agricultural Research Foundation.

² Professor of Plant Pathology.

TABLE 1.—MINIMUM AIR TEMPERATURES, SASKATOON, MAY, 1930.

Date	Minimum temperature	Date	Minimum temperature
May	°C.	May	°C.
12	5.3	16	-5.5
13	0.3	17	-0.1
14	-1.3	18	5.3
15	0.5		

TABLE 2.—DAILY RECORD OF METEOROLOGICAL DATA AT SASKATOON, MAY 17 TO 25, 1948.

Date	Maximum air temperatures	Sunshine	Evaporation	Wind
May	°C.	hr.	in.	M.P.H.
17	25.8	9.3	0.096	23.5
18	27.9	11.4	0.160	13.8
19	23.6	9.3	0.192	16.8
20	24.9	14.7	0.096	2.1
21	28.5	14.3	0.192	7.1
22	28.0	14.5	0.192	18.4
23	24.4	14.1	0.160	15.5
24	26.0	15.5	0.384	4.3
25	29.0	14.3	0.220	5.5

FROST BANDING OF SEEDLINGS IN 1930

Chlorotic banding of wheat and barley (Figure 1) seedlings with symptoms as described above were first observed by the author in May, 1930, at Saskatoon, following a period of successive nights when the minimum air temperatures, at the 4-ft. level, were around the freezing point (Table 1). Since the average minimum "grass" temperature for May, at Saskatoon, is about 1.6° C. lower than the corresponding air temperature, it is highly probable that the temperatures at the surface of the soil fell below the freezing-point on May 13 and 15. The effects of these four or five successive freezing temperatures at soil level on barley seedlings photographed on May 18 are shown in Figure 1. The banding was usually more conspicuous on barley than on wheat seedlings. A gradual greening of the chlorotic bands was observed to occur.

HEAT BANDING OF SEEDLINGS IN 1948

The wide distribution of chlorotic banding and general yellowing of cereal seedlings over considerable areas of north-central Saskatchewan has already been mentioned. A slight retardation in growth was suspected in fields showing the more severe types of injury.

On May 26, two samples of wheat seedlings with clumps of adhering soil were received from the Soils Department, University of Saskatchewan. One sample contained many seedlings which showed the chlorotic type of

banding with an occasional band of the bleached, dead type. This was collected from a loose, dry area of a silty, clay loam field. In the other sample the seedlings appeared normal and were collected from the same field where the soil was moist. The samples were collected on May 25. Meteorological data for May 17 to 25, 1948, are given in Table 2.

Both banded and normal seedlings were planted in separate flat boxes in the greenhouse and kept moist. The chlorotic bands of living tissue gradually turned a normal green colour, so that after five or six days in most instances it was difficult to detect the former position of the chlorotic bands. The bleached, white bands, however, failed to recover and leaves with this type remained broken over. In the field the tops of such plants would blow as flags in the wind.

The foregoing observations indicated that heat and not cold was responsible for the chlorotic banding of cereal seedlings in 1948, that dry surface favoured the banding, and that subsequent greening of the chlorotic bands occurred, but that the bleached bands failed to recover.

EXPERIMENTS ON HEAT BANDING OF CEREAL SEEDLINGS

Experimental Series I

The following preliminary experiments were conducted in early July to ascertain whether or not high temperatures were responsible for the banding.

Thatcher wheat was sown in boxes on the surface of moist soil, half of which were then covered with $1\frac{1}{2}$ in. of air-dried soil and half with moist soil. Half of each series was placed outdoors and half in a greenhouse with the glass roof lightly coated with a white wash.

Chlorotic banding developed only on the leaves of some seedlings in the outdoor boxes with the dry surface soil from one to four days after emergence. Those in the boxes with moist surface soil were normal green. Greening of the yellow banded seedlings occurred in the course of a few days. During the first few days after the seedlings emerged, the outside maximum air temperatures varied between 26° and 28° C., while in the greenhouse they were 5° to 8° C. higher. Afternoon surface-soil temperatures were around 45° C. in the boxes with dry surface soil in the open, and 4° to 11° C. lower in the boxes with moist surface soil.

These results indicated that direct insolation on dry surface soil was necessary for the production of heat banding on wheat and that high air temperatures alone, as occurred in the greenhouse, were not responsible. The cooling effect of evaporation from the surface of the moist soil kept the temperature below the critical point for chlorotic banding.

Experimental Series II

Further experiments were then conducted in the open to ascertain the surface-soil temperatures at which heat banding occurred. Wooden boxes, 12 in. \times 12 in. \times 10 in., were filled to within $1\frac{1}{2}$ in. of the top with a moist clay-loam field soil. Thatcher wheat was sown half an inch apart in rows 3 in. apart. The boxes were then filled to the top with air-dried soil, and were buried in the open so that their tops were level with the surface of the soil. Insolation and wind effect were much the same as in an open field.

In order that radiation effects would not be interfered with, all boxes were kept uncovered and the experiment repeated at intervals of a few days so that the chances of continuous fine weather with reasonably high maximum daily temperatures during the first few days after emergence would be met. Surface-soil temperatures were taken with ordinary glass centigrade thermometers placed at an angle of from 5° to 10° to the soil surface so that the mercury bulb was covered with a layer of soil from 2 to 3 mm. thick. There were two thermometers to each box. Temperature readings were recorded hourly from 12.30 p.m. to 3.30 p.m. from the day after the seedlings emerged until they were about four inches high. The highest average reading of the two thermometers of a box at a given time was taken as the maximum temperature for that box. The temperature variations in a given box were surprisingly low, seldom exceeding 5° C. It was felt that the method used for measuring the soil temperature was sufficiently accurate for the purposes of the study.

An experimental series, consisting of from two to four boxes, was set up on eight different dates during July and August. Four experiments, one of which included oats and barley, were successfully completed during periods of clear, warm weather; the remainder were spoiled by rain or cool, cloudy weather.

Daily records of maximum surface-soil temperatures in the experimental boxes and some meteorological data beginning with the date of seedling emergence for three experiments are given in Table 3. It will be seen that surface-soil temperatures of from 45° to 52° C. were recorded within an air temperature range of from 24° to 35° C. Figure 2 shows wheat seedlings from Series II, Experiment 1, with typical chlorotic heat-banding effects and the corresponding dates on which they were initiated. In this experiment, emergence began on July 31; the seedlings were removed for photographing at 11.45 a.m. on August 4. Chlorotic banding was the main type of injury in this experiment, with traces of whitespot burning at the tips and on one side of some leaves.

TABLE 3.—DAILY RECORD OF MAXIMUM SURFACE-SOIL TEMPERATURE AND OF METEOROLOGICAL DATA AT SASKATOON, 1948.

Date	Maximum surface soil temperatures	Maximum air temperatures	Sunshine	Evaporation	Wind
SERIES II.	$^{\circ}$ C.	$^{\circ}$ C.	hr.	in.	M.P.H.
<i>Experiment 1.</i>					
July 31	41 - 45	23.3	14.2	0.160	10.6
Aug. 1	44 - 52	24.8	10.2	0.128	3.8
Aug. 2	45 - 52	24.3	14.6	0.096	8.5
Aug. 3	46 - 52	26.3	11.4	0.160	12.6
<i>Experiment 2.</i>					
Aug. 7	42 - 46	26.6	10.1	0.160	7.1
Aug. 8	39 - 43	26.6	11.4	0.124	9.1
<i>Experiment 3.</i>					
Aug. 17	45 - 50	28.3	14.1	0.096	5.5
Aug. 18	45 - 52	34.8	11.6	0.224	11.6

In Series II, Experiment 2, leaf damage was again predominantly of the chlorotic banded type with, in addition, traces of leaf-tip burning on August 7, and a single case of burning on one side of an otherwise chlorotic lesion on August 8.

Series II, Experiment 3, contained Montcalm barley and Victory oats as well as Thatcher wheat. Leaf-tip chlorosis was produced in all the cereals on August 17, when emergence was general; in addition, there was some tip burning in barley. On August 18, chlorotic banding was abundant and burned lesions moderate.

The results of the various series show that cereal seedlings from one to four days after emergence are susceptible to chlorotic banding when the surface-soil temperatures are at least from 42° to 45° C., while from 45° to 52° C. bleaching or burning of the tissues becomes more common, and collapse and death of a few seedlings may occur at from 52° to 54° C. No injury was observed on seedlings where the surface-soil temperatures were below 42° C. Greening or recovery of the chlorotic bands takes from three to ten days or more. Montcalm barley was more sensitive to heat injury than Thatcher wheat or Victory oats. It is interesting to note that Olson (8) found Montcalm barley to be more susceptible than Mindum and Renown wheats to spring-frost injury and slightly more so than Vanguard oats.

The types of heat injury produced on cereal seedlings between surface-soil temperature limits of 42° C. and 52° C. are virtually indistinguishable from those produced by temperatures at or near the freezing point (cf. Figures 1 and 2).

DISCUSSION

It is interesting, physiologically, that temperature extremes at the soil surface can bring about the same abnormal effects or symptoms on cereal seedlings. This is probably due to the effects of temperature on the mechanism of chlorophyll formation. Thus Lubimenko and Hubbenet (7) have shown that the greening process of etiolated wheat seedlings takes place between temperature limits of about 2° C. and 48° C. These points do not depend upon the seedlings' exposure to light. In the view of these workers, temperature is related to the greening process through its influence on the synthesis of leucophyll and its transformation into chlorophyllogen, both of which processes are dark reactions. The transformation of chlorophyllogen into chlorophyll is a photochemical reaction. In Eyster's (6) view, there is only one precursor of chlorophyll, protochlorophyll, which through photooxidation is transformed into chlorophyll. It appears that in chlorotic banding of the leaves of cereal seedlings, whether caused by low or by high temperatures, the mechanism, probably enzymatic, controlling the formation of one or more precursors of chlorophyll has been interfered with. This mechanism may take a few to ten days or more for complete recovery. Desiccation of the tissues may be one factor involved, as this is known to inhibit chlorophyll synthesis. Regardless of the number of precursors, it seems that the temperature extremes have strongly influenced the mechanism for the development of chlorophyll precursors, especially in delaying recovery.

As far as is known, the close similarity of the three symptom types produced on cereal seedlings by surface-soil temperatures just above the

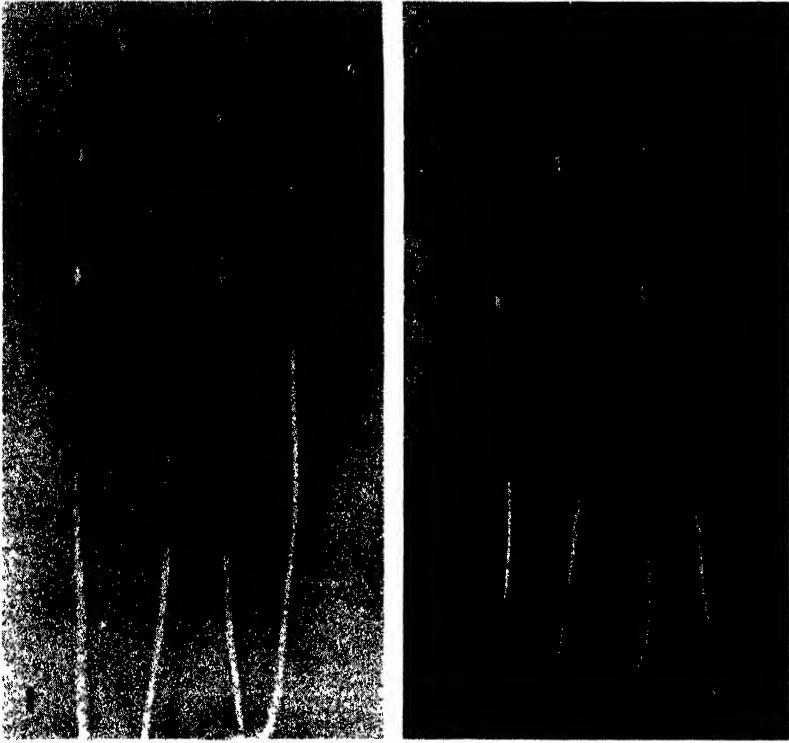


FIGURE 1. Chlorotic banding of fresh barley seedlings caused by slight frost at the soil surface on consecutive nights, May 13 to 17, 1930.

FIGURE 2. Chlorotic banding of dried wheat seedlings caused by heat (42° to 52°C.) at the soil surface on consecutive afternoons, July 31 to August 4, 1943.

minimum and below the maximum for their growth has not previously been pointed out. Attention is drawn to an analogous relationship between the similarity of some forms of "whitetip" or "ear tipping" damage of wheat spikes caused by frost and that caused by heat and drought. This similarity has previously been pointed out by Bell (1) and others.

Reddy and Brentzel (9) consider the critical temperature for heat canker of flax to be at about 54° C. They quote Mayr, and Münch, for the fact that the death point for vegetative cells lies at about 54° C. It seems probable, therefore, that the actual temperatures in the tissues of the seedlings in the experiments reported here may be slightly higher than the surface-soil temperatures recorded by the mercury thermometers.

Unless soil moisture is favourable, late-sown spring cereal seedlings on the Prairies are liable to injury from high surface-soil temperatures. A slight increase in the rate of sowing for late-sown cereals should be a good precautionary measure.

SUMMARY

Chlorotic banding, whitespot banding, and injury similar to damping-off were produced on spring-sown cereal seedlings by high surface-soil temperatures one to four days after emergence. Chlorosis first appeared when these temperatures registered from 42° to 45° C. (108° to 113° F.); whitespot injury increased as the temperature rose to 52° C. (125° F.); and collapse and death occurred at about from 52° to 54° C. (125° to 129° F.) and higher. These soil temperatures prevailed on clear summer days when air temperatures ranged from 24° to 35° C. (75° to 95° F.). Greening or recovery of the chlorotic bands took a few to ten days or more. The whitespot or bleached lesions did not recover, but later development of affected seedlings was normal. Injury to the growing points resulting in collapse was fatal. The same three types of symptoms are commonly found on seedlings which have been subjected to surface-soil temperatures at or near the freezing-point. Thus the same physiological effects on the development of chlorophyll and its precursors in spring-sown cereal seedlings can be produced by temperatures at or near the minimal and maximal cardinal points for growth. Slightly higher rates of sowing for late-sown cereals should insure against reduction in stand by heat.

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SULFATHIAZOLE IN RELATION TO AMERICAN FOULBROOD¹

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The use of sulfathiazole in the treatment of American foulbrood and the controversies over "pros" and "cons" have received much publicity in the trade journals. Comparatively little has appeared on this subject in the scientific literature based on carefully controlled experiments. What has appeared, however, has demonstrated beyond question the prophylactic and therapeutic effect of sulfa drugs on American foulbrood. At the same time, practically every scientific publication reports recurrences of infection, in some cases as high as 30 to 50 per cent, when treatments with sulfa syrup are discontinued. In consequence, a host of problems have presented themselves—the resistance of American foulbrood spores to these drugs, the influence of these drugs on the vegetative cells of *Bacillus larvae*, the etiological agent, the possible adaptation of the cells to the drugs; and in the apiary, continuous versus restricted feeding of medicated syrup, spraying of the drugs, adulteration of surplus honey, installation of package bees, masking and spread of the disease, period of isolation of infected, treated colonies, etc. As the following brief review of the literature will show, some of these problems have been resolved, others are being or remain to be studied.

Haseman and Childers (7) first reported that the feeding of sulfa drugs in sugar syrup or pollen substitute to bees could prevent the development of American foulbrood and that it resulted in the cleaning up of infected colonies. They claimed too that sulfathiazole "has a very beneficial action on bees infected with the *Nosema* parasite"—an observation which has not as yet been verified. Certain badly infected American foulbrood colonies were not cleaned up completely and when feeding was discontinued for two months, the disease reappeared in the late brood; these colonies finally died. In a later paper Haseman (5) reported that sulfaguanidine is as effective as sulfathiazole (and sulfanilamide). Furthermore, he stated that a thorough clean-up with sulfa may protect a colony against re-infection for two seasons but advised feeding the drug each spring and fall, or whenever infection appeared, as part of a regular apiary routine. In his most recent publication (6) he stated that streptomycin and penicillin may be used successfully but involve too much trouble.

Milne (13, 14) obtained essentially similar results but noted recurrence within 6 weeks of cessation of feeding. More important still, he reported that some colonies did not react successfully to treatment. Out of 32 colonies treated, however, 29 showed no evidence of disease during the period of treatment (33 to 148 days). Johnson and Stadel (11) and Johnson (9, 10) reported favorable results with 6 out of 7 colonies (among others) variously infected; three showed recurrence in the following season. Five out of 6 hives remained free of disease for two full years after the original treatment

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with sulfa, and 10 out of 11 passed through one year without a definite recurrence. Penicillin, furacin and x-ray treatment were ineffective; sulfaguanidine was much slower than sulfathiazole, but sulfadiazine and sodium sulfathiazole were as effective as sulfathiazole. Eckert (2, 3), using sodium sulfathiazole, observed recurrence with 2 out of 19 colonies, although further treatment resulted in the elimination of all visible signs of disease from these two colonies; the remaining colonies reacted favorably, some within a short time, wintered well and were clean the following season. Eighteen other infected colonies were successfully treated with sulfa and remained apparently clean throughout the season. He also reported that European foulbrood, sacbrood or paralysis of bees were not controllable with sulfa drugs.

In laboratory studies on the influence of sulfa drugs on *B. larvae*, Katznelson (12) showed that different strains responded differently to various concentrations of sodium sulfathiazole but that most were killed in a 1 per cent solution. This concentration did not affect the spores when suspended in water or 75 per cent honey for over 16 months. The organisms could be adapted to grow in 1 per cent of the drug but lost their resistance rapidly on being subcultured in absence of the drug. Certain antibiotic agents such as penicillin and notatin inhibited *B. larvae* strains in dilutions up to 1:5 or 1:10 million. The results reported herein represent experiments on the effect of sodium sulfathiazole on American foulbrood in the apiary.

EXPERIMENTAL

In the spring of 1948 experiments were set up to study both the prevention of infection and the cleaning up of infected colonies by sodium sulfathiazole. The drug was fed in the usual manner (from an inverted, perforated honey pail placed directly over the brood combs) at the rate of 0.5 gm. per gallon of a 1:1 sugar-water syrup; one gallon only was used per colony. American foulbrood spores were fed in the same syrup at the rate of about 2 billion per gallon. Each treatment was carried out in duplicate. In addition, p-aminobenzoic acid, the most potent sulfa-drug antagonist known (8) was fed to other colonies with and without sulfa at the rate of 0.5 gm. per gallon. At the same time, another experiment was carried out

TABLE 1.—INFLUENCE OF SODIUM SULFATHIAZOLE ON INFECTION OF BEE LARVAE WITH AFB SPORES

Treatment of 50 per cent sugar syrup April 4, 1948	Results
1. AFB spores	Heavy infection
2. " " + PABA*	Heavy infection
3. " " + sodium sulfathiazole	No infection
4. " " + PABA + sodium sulfathiazole	Infection
5. AFB spores kept in 1 per cent sodium sulfathiazole solution in water or honey for 4 months	Infection
6. No treatment	No infection
Infected colonies from treatments 1, 2, 4, 5, fed drug June 1-15—last examination Oct. 6	No infection

* p-aminobenzoic acid

TABLE 2.—RESULTS OF EXPERIMENTS ON SULFA FEEDING WITH SIX COLONIES OVER A PERIOD OF THREE YEARS*

Colony	1946			1947			1948
	June treated	August examined	Fall treated	June examined	June treated	Fall examined	June and fall examined
117	Sulfa	—	Sulfa	—	0	—	—
73	"	—	0	—	0	—	—
125	"	—	0	—	0	—	—
17	"	—	0	+	Sulfa	—	—
97	"	+	Sulfa	+	"	—	—
53	"	+	0	+	"	—	—

* Sulfathiazole or sodium sulfathiazole used.

0 = No treatment

+ = Infection

— = No infection

to determine the influence of the drug on the virulence rather than the viability of American foulbrood spores. Burnside (1) has reported that American foulbrood spores may be heated to a point where their virulence is destroyed but not their ability to germinate and produce growth in a suitable medium. Spores were kept in a 1 per cent solution of sodium sulfathiazole in both water and honey; they were then washed several times to remove the sulfa and fed in 50 per cent syrup. The results given in Table 1 show clearly that the drug prevented infection of the larvae and that p-aminobenzoic acid neutralized its action, thereby permitting infection to develop. P-aminobenzoic acid itself had no effect on American foulbrood spores nor did their immersion in 1 per cent sodium sulfathiazole solution for four months prior to testing.

All colonies showing infection were then given 1 gallon of medicated syrup and within 6-8 weeks they showed no sign of disease, remaining clean up to the last examination on October 6, 1948. Naturally it required a longer period for the badly infected colonies to be cleaned up. The infected material removed by the bees is dropped largely in front of the hive and constitutes a source of future infection of the colony although the material may be dispersed by rain or melting snow.

Another series of experiments had been set up in 1946*. Ten colonies were infected with American foulbrood combs after which two received sulfathiazole in tablet form, two sulfathiazole in powder form and two sodium sulfathiazole; three received penicillin and one was left as control. Penicillin failed to check the disease which developed rapidly; these colonies were then fed sulfa syrup and the disease began to disappear and healthy brood to become established. The results with the sulfa drug and subsequent manipulations of the colonies are given in Table 2. Four of the six colonies were cleaned up but two contained a few scales at the end of the season. Sulfa was fed as indicated but in June, 1947, 3 of the 6 colonies, or 50 per cent showed evidence of disease. However, when sulfa was again fed to the

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infected colonies, all evidence of the disease disappeared and all 6 colonies were apparently free from it throughout the 1948 season. These colonies are to be kept under observation.

Sulfathiazole was also tested in co-operation with a commercial beekeeper who in June, 1946, placed a total of 27 infected colonies at the disposal of the Bee Division. Medicated syrup was fed throughout the season and on September 25 only two colonies showed signs of the disease. Treated syrup was fed for the winter and again in the spring of 1947 and the colonies remained free from all signs of American foulbrood during this and the 1948 season.

DISCUSSION AND CONCLUSIONS

The results presented above indicate that American foulbrood may be controlled with sulfathiazole when used carefully by experienced workers. Whether the rank-and-file beekeeper can do this remains problematical. The human element was perhaps chiefly responsible for the failure of other promising treatments for American foulbrood; but even under the controlled conditions in the experimental apiary, recurrences of the disease in sulfa-treated colonies were observed. It is by no means certain that the six colonies listed in Table 2, though apparently free from disease, will not show evidence of it next year or the year after. Consequently, treatment with sulfa drugs may prove a long-term matter involving considerable time, labor, anxiety and uncertainty. It is in part for this reason that according to Eckert (2) "Most of the commercial beekeepers in California prefer to destroy the occasional diseased colony".

The masking and spread of the disease is one of the most serious considerations involved in the use of these drugs. The apparent disappearance of all visual signs of the disease may lead to careless handling on the part of the operator resulting in contamination of apiary equipment such as extractors, in the mixing of parts from infected hives with those from disease-free hives, in the mishandling of infected honey, and so on. For these reasons it has been suggested that sulfa treatment should be supervised by experienced personnel and that the infected treated colonies be isolated and placed in quarantine. The State of Florida (4) was the first to modify (not remove) its burning law to incorporate this treatment but only with the above provision.

It appears likely that sulfa drugs may play a definite though perhaps not a decisive role in the control of American foulbrood. It may be particularly useful as a preventive measure in areas where foulbrood abounds, for swarms of unknown origin and for package bees. The experimental work to date certainly warrants further extensive long-range trials by proper authorities.

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PREVENTION OF EARLY DECAY OF CUT POTATO SETS BY CHEMICAL TREATMENT¹

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It is a general practice to plant potato sets without treating their freshly cut surfaces with a fungicide as a protection against soil-borne pathogens. Whole potato tubers are often disinfected before being cut in order to kill such superficial pathogens as *Actinomyces scabies* (Thaxter) Güssow, *Rhizoctonia Solani* Kühn, and certain *Fusarium* spp. However, this treatment gives the cut surfaces of the sets no direct protection against the common soil-borne pathogens *Erwinia carotovora* (Jones) Holland, *Fusarium coeruleum* (Lib.) Sacc., and *Pythium ultimum* (Trow.), all of which are known to occur in certain cultivated soils in Alberta and elsewhere. According to present evidence, *E. carotovora*, which is usually prevalent, often produces incipient rot in cut sets if they are stored too long in sacks prior to planting. This infection, and those that develop during the next 15 to 20 days after planting, may destroy the set or greatly reduce its propagative value, resulting in a weak plant. In an unfavourably wet soil such damage can be very severe.

According to Wollenweber (9), *F. coeruleum* will produce severe rot of potato tubers at temperatures ranging from 15° to 28° C. In the present study, this pathogen was found to destroy potato sets within a period of 21 days in soil held at a temperature of 61° F. *F. coeruleum* has been isolated in this laboratory many times from rotting potato sets collected from recently planted fields, as well as from rotting tubers obtained from commercial fields and from places of storage in Alberta. Although *Fusarium sambucinum* Fkl. f. 6 Wr. has often been isolated from certain lots of tubers rotting in storage in Alberta, this pathogen seems to be of little importance in the decay of cut sets in the field.

Under average field conditions in Alberta, *P. ultimum* appears less important than either *F. coeruleum* or *E. carotovora* in the rotting of freshly cut potato sets. However, Jones (4) states that in British Columbia *P. ultimum* causes extensive rotting of potato sets in certain heavy soils.

The present study was made to determine if some of the standard as well as certain of the new commercial fungicides when applied to the cut surface of potato sets before planting would protect them adequately against rot by common soil-inhabiting pathogens, particularly *F. coeruleum*.

MATERIALS AND METHODS

The various tests were made in untreated Edmonton black loam, the pH value of which is approximately 6.1. Although some of the trials were made in virgin soil, the majority were in soil from a field cultivated about 20 years, and in this paper it will be referred to as bin soil. The water content of the soil was maintained at approximately 23 per cent (dry), 30 per cent (optimum), or 34 per cent (wet) m.h.c., as required in

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the various experiments, and the soil temperature was maintained at 61° F. The soil in which the potato sets were placed for comparing the protective value of the various chemicals was artificially infested with soil-grown inoculum of *F. coeruleum*, and, in one trial, *P. ultimum*. The effectiveness of each chemical was tested on 30 potato sets of uniform shape and weight, cut from healthy tubers of the Warba variety with a vegetable baller. These sets were well mixed to provide, as far as possible, representative samples for experimental use. Unless otherwise stated, the sets were cut and immediately treated with the various chemicals as required, and buried at regular intervals in infested soil, where they remained for a period of 21 days. At the end of this period they were taken up, cut in halves at right angles to the periderm surface, and rated for the degree of rotting present.

The following chemicals and proprietary compounds were tested: mercuric chloride, yellow oxide of mercury, sulphur, calcium hydroxide, Semesan Bel (nitrophenol mercury + chlorophenol mercury), Dithane 14 (disodium ethylene bisdithiocarbamate), Roccal (10 per cent alkyl-dimethyl benzyl ammonium chloride), Fermate (ferric dimethyl dithiocarbamate), Lunasan (ethyl mercury thiourea), Ceresan (5 per cent ethyl mercuric phosphate), and Spergon (tetra-chloro-para-benzoquinone). They were applied to the sets in the form of a solution, suspension, or as a dust, as indicated in Tables 1 and 2.

EXPERIMENTAL RESULTS

Experiments 1, 2 and 3

The purpose of Experiments 1, 2, and 3 was to ascertain whether or not freshly cut potato sets, when immersed in a solution of mercuric chloride (standard and acidified), or of Semesan Bel, are afforded a satisfactory degree of protection against attack by soil-borne *F. coeruleum*; and if any such protection is modified by the water content of the soil or by delay in the planting of the sets. Although the above mentioned chemical treatments are now used on whole tubers without serious injury to the vigour of the sprouts, very little is known regarding their effect on the natural healing processes of the freshly cut surface of a set, especially at lower temperatures in a soil which is unfavourably dry, or in one too wet. It is obvious that the potato set should have full protection immediately it is planted, since after about 15 to 20 days it will have established an independent plant.

According to data from the various tests in Experiments 1 and 3 (Table 1), the freshly cut sets treated with mercuric chloride or Semesan Bel were not satisfactorily protected against attack by *F. coeruleum*, although some benefit was usually evident. In the wet soil series of Experiment 2 (Table 1), there seems to have been practically no protection of the treated sets against a chance contamination of them or of the soil by *E. carotovora*. The untreated sets of the control in this soil series apparently escaped.

On the basis of the data for the untreated sets of the controls in all three experiments (Table 1), the sets in the dry soil series were more severely rotted than those in the wet soil, and particularly so in Experiment 3,

TABLE 1.—DEVELOPMENT OF ROT IN TREATED CUT POTATO SETS IN NATURAL WET AND DRY SOIL¹, ARTIFICIALLY INFESTED WITH *Fusarium coeruleum*. THE SOIL WAS HELD AT 61° F. DURING THE 21-DAY INCUBATION PERIOD

Treatment	Time	Experiments							
		No. 1		No. 2		No. 3			
		Wet soil	Dry soil	Wet soil	Dry soil	Wet soil		Dry soil	
						A ²	B ²	A ²	B ²
	Min.	%	%	%	%	%	%	%	%
Mercuric chloride (1-1,000, plus 1 per cent hydrochloric acid)	5	5	28	86	46	38	28	30	25
	1	18	50	99	32	24	32	38	38
Mercuric chloride (1-1,000, not acidified)	5	16	6	62	24	21	24	42	44
	1	18	11	62	23	25	39	16	51
Semesan Bel (as recommended)	5	17	10	63	24	35	35	44	42
	1	15	21	43	31	7	10	41	27
Average of treatments	—	14.8	21	69	30	25	28	35.1	37.8
Sets not treated	—	40	62	19	38	37 ⁴	37 ⁴	63 ⁴	84 ⁴

¹ Approximately 34 per cent and 23 per cent m.h.c., respectively.

² Sets cut and planted immediately.

³ Sets cut and held for 24 hours at 61° F.

⁴ Based on 180 sets.

where the averages given are based on 180 sets, instead of on 30 as in Experiments 1 and 2. However, there was no significant difference in disease development when the sets were treated.

The data obtained on the effect of planting the treated cut sets immediately, or holding them at 61° F. for 24 hours, indicate no marked trend. However, in the untreated sets planted in dry soil, there appeared to be a difference of 21 per cent in favour of planting them immediately, but none when the water content of the soil was slightly above optimum.

From the evidence in Table 1, it appears that, in general, the treated cut sets were rotted slightly less severely by *F. coeruleum* than the untreated sets, especially when the latter were planted in a dry soil or held for some time before they were planted. However, it is clear that none of the chemical treatments employed gave sufficient protection to recommend their use in field practice.

Experiments 4, 5, 6 and 7

The results of the first three experiments (Table 1), indicated that further trials should be made with other chemicals. Four additional experiments were carried out at different periods. The water content of the soil in these experiments was maintained at approximately optimum, namely 30 per cent m.h.c. The data on the effects of the various chemicals tested on disease control and on sprout vigour are summarized in Table 2.

Under the conditions of these experiments, the sulphur, calcium hydroxide, Dithane 14, yellow oxide of mercury, and Roccal treatments gave very limited protection to the potato sets against attack by *F.*

TABLE 2.—SEVERITY OF ROTTING OF TREATED FRESHLY CUT POTATO SETS IN A SOIL ARTIFICIALLY INFESTED WITH *Fusarium coeruleum* OR *Pythium ultimum*

Treatment	<i>Pythium ultimum</i>	<i>Fusarium coeruleum</i>				Vigour of Sprouts
	Experiment No. 4 Bin soil	Experiment No. 4 Bin soil	Experiment No. 5 Bin soil	Experiment No. 6 Virgin soil	Experiment No. 7 Virgin soil	
	%	%	%	%	%	
Sulphur (dust)	89 *	91 *	—	—	—	Average
Fermate (dust)	0	0	0	—	0	Average
Fermate, 1 per cent	32	87 *	—	—	—	Average
Dithane, 0.4 per cent	40	68	—	—	—	Average
*Yellow oxide of mercury, 0.4 per cent	—	33	27	—	—	Average
Calcium hydroxide (dust)	—	—	38	—	—	Average
*Roccal, 1 per cent	—	—	17	—	—	Average
*Lunasan, 1 per cent	—	—	0	—	—	Stunted
Lunasan, 0.5 per cent	—	—	1	—	—	Weak
*Ceresan, 1 per cent	0	0	0	—	—	Dormant
Ceresan, 0.5 per cent	—	—	0	—	—	Dormant
*Spergon (dust)	—	—	0	0	0	Strong
Spergon, 2 per cent	—	—	—	0	0	Strong
Spergon, 1.5 per cent	—	—	—	0	0	Strong
Spergon, 1 per cent	—	—	—	1	Trace	Strong
Untreated sets	67	80 *	53	82	26	Average
Uninfested soil, untreated sets	—	—	10	0	0	Average

* Optimum water content 30 per cent m.h.c., and temperature 61° F.

* Dip treatments showing percentage of active ingredients per litre of 50 : 50 Aerosol—water (except Roccal).

coeruleum. On the other hand, Fermate applied as a dust, Lunasan and Ceresan applied as dip treatments, and Spergon applied either in dust form or as a 50 : 50 water-Aerosol solution containing as low as 1 per cent active ingredients gave practically 100 per cent protection to the sets (Figure 1A).

With reference to the soil series infested with *P. ultimum* (Experiment 4, Table 2), sulphur was useless; the Dithane and Fermate dip treatments were only partially effective; but Fermate applied as a dust, and Ceresan as a dip treatment, afforded the sets apparently complete protection. Spergon was not included in this series.

With reference to the effect of the various chemicals used in these experiments (Tables 1 and 2) on the vigour of the sprout of the treated set, certain important differences were observed. The sprouts from sets treated with Spergon seemed uniformly above normal in vigour in all tests. In contrast, sprouts were not produced by the sets treated with Ceresan, and they were stunted or weak in the Lunasan treatment. None of the remaining chemical treatments consistently reduced sprout vigour, although occasionally both Semesan Bel and mercuric chloride seemed to be detrimental.

Finally, attention is directed to the typical contrast (Figure 1B) in soundness and colour of the untreated sets incubated in the "uninoculated" control series of the virgin and cultivated soils used (Table 2, Experiments 5 and 6). Numerous spores of *Fusarium* spp., evidence of other fungi, and

many starch grains were found in the decaying tissue of the sets. Consequently, it is suggested that unprotected sets in an infested soil could be a fruitful source of inoculum potentially dangerous to the new crop of potato tubers during harvesting operations and later in storage.

DISCUSSION

Cunningham and Reinking (3), in their studies of *Fusarium* seed-piece decay of potato on Long Island, U.S.A., feel that the treatment of whole tubers is best suited to farm practice because they can be treated when convenient and stored until cutting time. However, they warn against contamination of the freshly cut sets and stress that, if these must be held any length of time before being planted, they should be stored under conditions favourable for rapid healing of their cut surfaces. Under average farm conditions, the freshly cut sets are very likely to be contaminated with pathogenic fungi and bacteria. In Alberta, the cut sets are often held in storage at temperatures around 60° F., or lower, during several days. Even if they are planted the same day as cut, the temperature of the soil at this time of the year is more likely to be less than 60° F. than higher.

According to Priestley and Woffenden (5), wound periderm in potato tubers may not appear until 12 to 15 days at 61° F. Also, several investigators (1, 2, 5) have found that the healing processes, namely suberization and wound periderm formation, develop more slowly in certain varieties than in others. In a separate study, the results of which will be reported later, wound periderm appeared in about 10 days in cut potato sets buried in moist soil at 61° F., whereas the blocking-off process of the cut surface apparently was not well advanced before the end of three days.

Weiss, Lauritzen and Brierley (8) concluded from their comprehensive infection studies that at 61° F. the two or three layers of cells immediately below the cut surface which become suberized (2) required fully three days to heal well enough to resist penetration by *F. coeruleum* effectively. Successful fungal infection during this vulnerable period could arrest the natural healing processes.

The experimental data presented in Table 2 show that at 61° F. freshly cut potato sets, planted immediately in a natural soil of optimum water content and artificially infested with *F. coeruleum*, were very severely rotted (Figure 1A) within a period of only 21 days. This could happen in the field and be accentuated by bacteria causing soft rot. It was also shown in Table 2 and Figure 1A that, under the same conditions, the sets remained sound when their cut surfaces were treated with Fermate applied as a dust, Lunasan and Ceresan applied as dip treatments, and Spergon applied either as a dust or a dip. The degree of control obtained from Semesan Bel, Dithane, yellow oxide of mercury, mercuric chloride, sulphur, and calcium hydroxide was inadequate for the purpose intended. Although none of the chemicals in this latter group obviously reduced the vigour of the sprouts, those containing mercury were suspected of lowering sprout vigour. The sprouts were quite weak to stunted when the sets were treated with Lunasan, but wholly suppressed by Ceresan. They seemed uniformly stronger than normal on the sets treated with Spergon.

The foregoing data seem to indicate that, under field conditions, highly beneficial results of commercial importance may be obtained by treating freshly cut potato sets with certain chemicals.

It may be asked if the treatments suitable for controlling rot by *Fusarium* spp. in cut sets would be as effective in reducing the development of common scab and stem canker as those now recommended for whole tubers. The answer is not available from the data of the present study, but could be obtained by making controlled tests of promising chemicals in the laboratory. Excepting when the surface of treated whole tubers is heavily infested with scurf or sclerotia of virulent races of *R. Solani*, the detection of a difference between the two methods in disease control would be difficult in field culture (6, 7).

SUMMARY

The value of various chemicals for protecting freshly cut potato sets, planted in field soil, against attack by *Fusarium coeruleum* was compared in controlled laboratory experiments. The soil was artificially infested with the pathogen, maintained at a temperature of 61° F. and a water content (30 per cent m.h.c.) about optimum.

Untreated sets were severely rotted within 21 days, but when the sets were dusted with Fermate, or when Semesan or Lunasan were applied as dip treatments, or Spergon used as a dust or applied as a dip (1 per cent active ingredient), they remained sound. Sets treated with Semesan Bel, Dithane, yellow oxide of mercury, mercuric chloride, sulphur, or calcium hydroxide were afforded inadequate protection.

Apparently the pathogen rotted untreated sets more when the soil was rather dry (23 per cent m.h.c.) than when fairly wet (34 per cent m.h.c.).

Excepting Ceresan and Lunasan, the chemicals used were not obviously detrimental to sprout vigour. Ceresan suppressed the sprouts and Lunasan stunted them. Spergon seemed to increase sprout vigour.

ACKNOWLEDGMENT

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A NOTE ON THE CAUSE OF BRITTLE DWARF OF WHEAT

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The name Brittle Dwarf was suggested by Fraser *et al.* (2) in 1932, for a disease of winter and spring wheats characterized by a stunting and extreme brittleness of the culms, usually accompanied by a proliferation of the shoots and yellow mottling and streaking of the leaves (Figures 1 to 4). Other occasional features were malformed heads, distorted peduncles, and twisted terminal leaves. Aphids were usually associated with the diseased plants and were frequently present in large numbers under the leaf sheaths and in twisted leaves. The trouble appeared to spread from an infection source; it occurred both in patches and on isolated plants. The plots of spring wheat affected with brittle dwarf were adjacent to the affected winter wheat plots, though this is not mentioned in the first communication (2). Seed transmission was not obtained experimentally. H. H. McKinney, U.S. Dept. of Agriculture, to whom specimens were sent, concluded that it was not the same as the mosaic of wheat (7) occurring east of the Mississippi River.

Since 1932 the disease has been reported several times on wheat in various parts of Saskatchewan, being usually found at the edges of fields. In one field, 25 per cent of the culms were affected in a patch 50 feet in diameter (4). It was first recorded on barley by W. G. Sallans and R. J. Ledingham (3) in 1933, in an experimental plot in which 7 per cent of the plants were affected. Records of the disease have also been made by entomologists (1). Renewed interest in the disease was recently aroused by Simmonds (5) in 1947. Affected wheat and barley plants were observed scattered through his experimental plots. A. P. Arnason of the Dominion Laboratory of Entomology pointed out to him the similarity of injury on nearby crested wheat grass (*Agropyron cristatum* (L.) Gaertn.) caused by aphids, and that on the wheat and barley, and also drew to his attention a similar trouble described on winter wheat by Parker (8) in 1916, in Montana, as being caused by the western wheat aphid (*Brachycolus tritici* Gill.). Simmonds suggested that the serious nature of the outbreak on one of our common forage grasses pointed to the necessity of both entomologists and plant pathologists studying the etiology of the disease. The increasing production of winter wheat in southern Alberta and south-western Saskatchewan adds further weight to this contention.

No indication of the presence of nematodes in diseased plants could be found so that any similarity to the symptoms of the disease of wheat caused by *Tylenchus tritici* (S.) Bast. (6) was merely fortuitous. No cell inclusions were observed in mottled or streaked leaves. Experiments had shown that the trouble was not seed-borne. These two facts ruled out mosaic or rosette (7) as being concerned. A bacterial disease associated with some affected plants in 1925 and 1928 was believed to be black chaff (2). It was later found that these plants were hybrid material, very

¹ Contribution from the Laboratory of Plant Pathology, University of Saskatchewan, Saskatoon, Sask. with financial assistance from the Saskatchewan Agricultural Research Foundation.

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susceptible to black chaff (*Xanthomonas translucens* f. sp. *undulosa* (E.F. Sm., L. R. Jones, and Reddy) Hagb.). No evidence remained suggesting a pathogenic relationship to the disease. The main evidence in support of the virus hypothesis was that affected plants were sometimes found on which aphids were very few or entirely absent. Final speculation as to the cause was twofold: Was the injury due wholly to aphids, or were they also serving as vectors of a virus?

EXPERIMENTAL

Preliminary experiments were conducted in 1931 and again in the autumn of 1947¹ to test the transmissibility of brittle dwarf by the wheat aphid. In the earlier tests aphids were taken from affected wheat plants and allowed to feed on healthy wheat seedlings for one or two days; in the later experiments the aphids² were taken from both affected wheat and crested wheat grass plants. No evidence of insect transmission of the disease was obtained.

In late June, 1948, severe brittle dwarf developed on spring-sown perennial wheat plants (*Agropyron elongatum* Host \times *Triticum vulgare* Vill. var. Chinese) adjoining pots of overwintered perennial wheat (Figures 1 and 3). Aphids from affected plants were used to inoculate healthy wheat seedlings.

POTS

Thatcher wheat was sown in 6-in. pots, three seeds to each pot, and when the seedlings were 4 in. high an experiment comprising three series as outlined below was set up. Three such experiments were conducted during July and August; the first began on July 14 and the others at about weekly intervals thereafter.

Series I.—Aphids were allowed to feed on one pot of seedlings for 24 hours, and on another pot for 48 hours. After removal of the aphids, the pots were then placed in a fine-meshed wire cage (28 mesh to the inch) and sprayed with nicotine sulphate solution. On the same evening, the plants were fumigated with Nicofume.

Series II.—Two pots of seedlings were similarly inoculated with aphids and placed under screens in an adjoining compartment of the greenhouse. The aphids were reproducing parthenogenetically and in the course of a few days the plants were heavily infested.

Series III.—Two pots of seedlings were kept free of aphids.

Seven weeks after the first experiment was set up, the plants in Series I and III had headed out, averaged 20 to 21 inches in height and showed no signs of brittleness. Those in Series II were about 7 inches high, brittle and severely mottled and streaked. Symptoms as severe as these are sometimes encountered in the field (Figure 3).

¹ The 1947 experiments were conducted by W. M. Dion, to whom thanks are due for permission to include her results.

² A. P. Armason kindly identified these as the western wheat aphid (*Brachycolus tritici* Gill.).



FIGURE 1. A typical portion of a row of spring-sown perennial wheat affected with brittle dwarf. The "escaped" culms may be slightly brittle.



FIGURE 2. Mottled and streaked leaves, a distorted head and a crinkled and twisted leaf.



FIGURE 3. Two plants with severe symptoms—stunting, conspicuous yellow mottling, extreme brittleness, and empty heads.

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FIGURE 4. Spring wheat plants affected with brittle dwarf.

The results of the two other similar experiments were virtually the same. In Series II, care had to be exercised so as not to allow the infestation to become too heavy; otherwise the plants would be killed before becoming brittle.

The foregoing experimental results give no indication that a virus is associated with the disease. They point strongly to the conclusion that the so-called brittle dwarf disease of wheat, barley, crested wheat grass and other grasses is caused by the western wheat aphid.

Parker (8) has mentioned the severe damage which can be caused by this aphid and our observations in Saskatchewan bear this out in limited areas. He found, however, that during the hottest summer weather, coccinellid and hymenopterous parasites greatly reduce the numbers of aphids. This may explain why only localized outbreaks have occurred in Saskatchewan in the past. Practical recommendations for the control of this aphid may be found in Parker's paper.

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CONCERNING THE MOVEMENT OF THE BULB EELWORM, *DITYLENCHUS DIPSACI* (KUHN) FILIPJEV, IN NARCISSUS BULBS¹

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In his description of the eelworm disease of narcissus, caused by the bulb eelworm *Ditylenchus dipsaci* (Kuhn) Filipjev, Goodey (1) states that the worms wander down into the tissue of the particular bulb scale which they first enter, and there produce a brown ring of diseased tissue, and that, as the disease advances, more and more of the fleshy scales become invaded until finally the whole bulb may be involved. However, he does not explain how the disease spreads to scales other than those first entered by the worms. Stillinger (2) states that the worms work between the cells, causing the disintegration of the middle lamella, and that the affected scales turn yellow or brown. He notes that infection may extend laterally, but he found that it advances more rapidly towards the base of the bulb and does not extend across from one scale to another. He concludes that, within the bulb, the spread of infection from scale to scale takes place by movement of the worms to the base and from the base to uninfected scales.

From these accounts, it may be inferred that the worms occur in the brown scales only, these scales forming the brown ring symptom typical of an eelworm infection. However, evidence has been secured that the bulb eelworm may also occur in the white scales lying between brown scales.

A lot of narcissus bulbs, variety Laurens Koster, was collected from the stock of a British Columbia bulb-grower on August 10, 1948. Many of these bulbs were soft as a result of eelworm infection, and a fair proportion of them carried eelworm-wool. Dissection of these bulbs showed that the sheaths of many uncoloured scales could be readily separated because of the disintegration of the middle lamella of the cells of the scales. Scrapings from the inner surface of these scales disclosed the presence of the bulb eelworm in all stages of development. Longitudinal strips of uncoloured scales were divided into quarters for examination. The eelworms found in the basal quarter were mostly pre-adults, but those in the apical quarter were mostly eggs and young larvae. Furthermore, a number of bulbs were cut across in the neck region, and the white scales lying between brown scales were examined. Eggs and young larvae and a small proportion of adults and pre-adults were frequently encountered.

A study was also made of a lot of narcissus bulbs, variety King Alfred. These bulbs arrived in British Columbia from Great Britain and were inspected on October 29, 1948. A small percentage of the bulbs was found infected with the bulb eelworm. Examination of the infected bulbs disclosed that the worms had moved through the initially infected scales into the basal plate, and from there had spread laterally to several consecutive scales. The brown discoloration was present only in the basal portion of the

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infected scales. The scales were divided horizontally into quarter-sections for examination. The eelworm population found in the brown basal section was dense, but in the next two non-coloured sections it was relatively sparse. No eelworms were found in the apical section. The embryo leaves which had grown almost level with the crown of the bulb were also found to be infected. Infection was confined to the lower half, and a slight brown discoloration had developed.

The data secured from the English bulbs support the conclusion of Stillinger (2) that the spread of infection from scale to scale takes place by way of the basal plate, and that the infected scales turn brown. In this instance, the brown discoloration was only in the basal quarter-section of the scale where the eelworm population was more dense than in the uncoloured sections. There appears to be a connection between the age of infection and the extent of discoloration. This conclusion is supported by the data from the two lots of bulbs under study. The eelworms in the British Columbia bulbs were distributed from the base to the apex of the infected scales, but the scales were not discoloured when these observations were made on August 10. On the other hand, the eelworms in the English bulbs were found in the basal section and also the next two sections of the infected scales. They were not found in the apical section. The basal section was the only one that was discoloured. These observations were made on October 29. It can be inferred that the eelworms moved more rapidly through the British Columbia bulbs than through the English bulbs, and consequently there was no discoloration in the British Columbia bulbs.

The evidence of rapid movement of the worms through the British Columbia bulbs supports evidence that eelworm-wool appears earlier and more frequently in local bulbs than in bulbs from England and the United States. In 1934, correspondence with the United States Division of Nematology indicated that eelworm-wool was not recorded in Washington State before early September or in New York State before late September. At that time, Hastings (3) had already reported that eelworm-wool was found in British Columbia bulbs on July 26. On the other hand, eelworm-wool was found on a lot of imported English bulbs on September 29. Recently (April, 1948) a letter from a nematode authority in Washington State stated that eelworm-wool does not form readily in that State in most years. The environmental conditions in British Columbia are apparently more favourable than in the United States, and possibly also in England, for the development of eelworm populations.

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SIZE OF SEED IN RELATION TO SIZE AND SHAPE OF ROOT IN SWEDE TURNIPS

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INTRODUCTION

Among the factors of quality in the regulations under the Seeds Act it is stated that seeds, within reasonable limits, shall be uniform. Uniformity in size, while it is taken into consideration in applying the regulations, is not precisely defined. The seeds of turnips may vary quite markedly in size but there is little information on the relationship between size of seed in this crop and yield. In February, 1946, this question was brought to the attention of the Division of Plant Products by a prominent grower of swede turnips. He contended that the smaller seeds were slower to germinate and consequently produced smaller roots than the larger seed and also that there was a greater variation in type of roots, when the seed used varied in size. Uniformity in type and size of root are important factors in marketing the crop.

At the same time this grower provided a sample of registered seed of one of the most widely grown varieties, Laurentian, to illustrate his contention. It was decided to conduct some trials on the plots of the Plant Products Division at Sackville, N.B., using this material and this was done during the summer of 1946. At the same time, tests of germination speed and germination capacity were carried out in Seed Research Laboratory, Ottawa. These are discussed in Part I of this paper. Since the findings of these trials seemed to run counter to such as had been noted in the literature, it was felt that a repetition of the work on a more extended scale should be undertaken. This was planned for the growing season of 1947 but could be carried out only in part owing to the destruction of some of the plots. This work is considered in Part II.

Part III deals with size of seed in relation to shape of root.

REVIEW OF LITERATURE

While a considerable amount of work has been done on size of seed in relation to plant development, root crops do not appear to have received as much attention as other types of crop. Findlay (4) in 1919, using various crop seeds, including turnip, studied the problem from two points of view, namely (a) different sized seeds in the same sample and (b) different sized seeds in different samples of the same kind of seed. The results with turnips showed slight, but consistent, increases in yield with the larger seeds of a given sample but small seeds of a good strain proved superior to

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large seeds of a poor strain. Results very similar to these have been obtained at the Ontario Agricultural College, according to a communication from G. P. McRostie. Other workers have investigated the effect of size of seed on yield and growth using a variety of crop plants other than roots (except beets, in which "seed", however, connotes the beet-ball and which therefore need not be considered here). Brenchley (2) concluded that with short-lived species there was an advantage in using heavier seeds but that the advantage became less marked, though still in evidence, with longer-lived species. A somewhat similar conclusion was drawn by Oexemann (7) who found that the importance of the seed weight factor was greatest in the early stages of development but the advantage became less as the plant matured and was lost at maturity. The mortality rate, however, was higher among seedlings from small seeds. His conclusion is similar to that of Kotowski (5) who found that size of seed influences the size of plant during the first 60 days but that the effect disappears after that time. Various Russian workers have studied the problem in grains (3, 9 and 10) and in cucurbits (6) and have concluded that the larger seeds give a greater yield.

As to speed of germination, Alessandretti (1) found a pronounced difference in favour of the smaller seeds although there was no difference between large and small seeds in germination capacity. He concluded that the only advantage of large seeds lay in their more voluminous food reserves from which deeply covered seeds might benefit.

Part I. 1946 Growing Season

MATERIALS

The sample as received consisted of a number of sub-samples of a lot of Registered Laurentian Swede as follows:

Lab. No.	Fraction
RL 140	Representing the bulk sample
A	Seed retained by a 1/14 screen
B	Seed retained by a 1/15 screen
C	Seed retained by a 1/16 screen
D	Seed retained by a 1/18 screen
E	Seed retained by a 1/22 screen

These screen sizes were represented in the bulk sample in the following proportions:

Screen size	Per cent of bulk sample
1/14	29.5
1/15	30.2
1/16	31.3
1/18	7.5
1/22	1.5
	<hr/> 100.0

In addition another sample of Laurentian Swede was grown in the plots at Sackville to serve as a check.

SPEED OF GERMINATION AND GROWTH

METHODS

Studies of speed of germination were made by a method developed in the Seed Research Laboratory which has proved very sensitive for several kinds of seed. As it has not previously been described, it is given here in some detail. It actually measures speed of germination and initial growth.

Each seed is germinated in a shell vial. For small seed such as the present, vials approximately 67 mm. \times 17 mm. are used. The vials are, however, carefully calibrated and selected as to diameter size so that the diameters in an entire series are as equal and uniform as possible ($\pm .01''$).

A measured volume of dry sand of rather uniform grain size (the exact size is not important within a range of about 0.5 mm. to 1.5 mm.) is placed in each vial and sufficient water added so that when shaken and tapped the sand is all wetted and has a thin film of water over the surface. Care must be taken that the surface is level. One seed is then planted as exactly as possible in the centre and a measured volume of dry sand poured on top, the surface being levelled by gentle shaking. The prepared vials are placed in a box containing dividing strips (similar to those in an egg-crate) and the whole placed in a controlled temperature cabinet. It is advisable to run a whole series in one cabinet because slight differences of temperature between two cabinets will materially affect the results.

In the present series the shell vials were 0.58" in diameter (internal), 5.3 cc. of sand were placed in each and 2 cc. of water added. The seed was covered with 2.6 cc. of dry sand. The tests were placed in a germinator at 20° C.

The germinating seedling almost invariably grows straight up the centre of the column of dry sand above it and it can easily be detected the moment it breaks surface. Readings are made as often as necessary during the period when surface penetration is occurring.

RESULTS

Sample E was not used in these tests since there would not then have been sufficient seed of this size for the field trials.

Twenty-five seeds each of samples A, B, C and D were planted as described, between 2.30 p.m. and 3.05 p.m., March 12, 1946. First penetration occurred at about 4.50 p.m., March 15. Table 1 gives the results.

TABLE 1.—SPEED OF GERMINATION—VIAL TESTS

Date	Time	Seedlings penetrated			
		A	B	C	D
March 15	4.50 p.m.	1	1	0	4
March 16	9.15 a.m.	14	17	14	10
March 16	11.35 a.m.	18	19	17	17
March 18	9.15 a.m.	25	25	23	23

Two seeds of each of C and D failed to germinate. Bearing this in mind there is little, if any, tendency on the part of the smaller seeds to lag in germination speed in relation to the larger.

The seedlings were carefully removed, washed and measured with the results given in Table 2.

TABLE 2.—AVERAGE LENGTHS OF SEEDLINGS—VIAL TESTS

Sample	No. seedlings	Mean length, cm.	S. D	S. D. (mean)
A	25	2.88	0.625	0.125
B	25	2.79	0.604	0.121
C	23	3.06	0.548	0.114
D	23	3.10	0.704	0.147

The greatest difference occurred between D and B, a difference of 0.31 cm. This gives $t = 1.63$. Since this is the greatest difference among six possible comparisons, $t = 2.8$ at the 5 per cent point. Thus the difference is not significant.

In a second series of tests, 50 seeds of each of samples A, B, C and D were planted between blotters and placed in a germinator at 20° C. These blotters were examined three times a day and the initiation of germination of each seed recorded.

The tests were planted at 3.30 p.m., March 18, 1946. Germination was completed by March 22 and at 2.35 p.m. of that date, hypocotyls of all seedlings were measured. Tables 3 and 4 give the results.

TABLE 3.—SPEED OF GERMINATION—BLOTTER TESTS

Date	Time	Seeds germinated			
		A	B	C	D
March 19	11.00 a.m.	1	5	2	2
March 19	3.00 p.m.	13	12	14	12
March 20	9.45 a.m.	47	50	47	43
March 20	4.30 p.m.	—	—	50	45
March 22	*	3	—	—	5

*Dead seeds or abnormal sprouts.

Table 3 agrees in general with Table 1. The 3 and 2 seeds which germinated in samples C and D, respectively during the day of March 20 are too few and the delay in germination too brief to be of significance in relation to the present problem.

In comparing Tables 1 and 3 it should be borne in mind that in Table 1 it is penetration of seedlings that was recorded; in Table 3 it is the first evidence of emergence of the radicle from the seed coat that was recorded.

TABLE 4.—LENGTHS OF HYPOCOTYLS—BLOTTER TESTS

Sample	No. of seedlings	Mean length, cm.	S. D.	S. D. (mean)
A	47	1.30	0.247	0.036
B	50	1.27	0.285	0.040
C	50	1.38	0.235	0.033
D	45	1.23	0.337	0.050

This accounts for the differences in elapsed time before the first records in the two tables.

The greatest difference in length of hypocotyl occurred between C and D, giving a *t*-value of 2.4. Since this is the greatest difference among six possible comparisons, the 5 per cent point for the value of *t* is about 2.7. Thus the difference is not significant and the blotter tests confirm the conclusion of the vial tests.

These tests show that the germination capacity was about 96 per cent.

SIZE OF ROOTS PRODUCED BY SEEDS OF DIFFERENT SIZES

METHODS

Each size of seed was planted in duplicate rows in two blocks on a level and uniform part of the experimental field at Sackville. The total weight of roots in each row was determined and each root was measured by means of a tree caliper graduated in tenths of an inch, the polar and equatorial diameters being recorded. Where the roots were not typically globe-shaped, which occurred in a few cases, two equatorial measurements were taken and these were averaged in the calculation of the approximate volumes as described below.

On an adjacent plot another lot of Laurentian swede was grown from which 300 roots were harvested and measured, for purposes of comparison.

A preliminary investigation of the raw data showed that it would be necessary to study the volumes if definite conclusions were to be drawn.

As an approximation to the volume of individual roots, the formula for the volume of an ellipsoid was used

$$V = \frac{4}{3} a b c$$

where *a*, *b* and *c* are the three semi-axes of the object. It was assumed that the roots were reasonably circular in cross-section so that half the figure for width would represent each of two of the semi-axes and half the figure for depth would represent the third. The data for each root were converted into volume and the resulting figures used in the analysis which follows.

The ratio: Mean weight/mean volume was calculated from the weights and volumes of the roots produced by each size of seed and these ratios are given in Table 5.

TABLE 5.—WEIGHT/VOLUME RATIOS

Sample	Ratio	Sample	Ratio
Bulk	0.0402	C	0.0394
A	0.0395	D	0.0376
B	0.0386	E	0.0375

These ratios are fairly constant, which suggests that the method of computing volumes should be satisfactory.

RESULTS

In Table 6 are presented the average weights and volumes together with the numbers of roots harvested.

TABLE 6.—MEAN WEIGHTS IN POUNDS AND VOLUMES IN CUBIC INCHES OF ROOTS—1946

Sample	Block 1			Block 2			Volumes, average of blocks
	No.	Means		No.	Means		
		Weight	Volume		Weight	Volume	
Bulk	81	2.48	60.9	44	4.41	111.1	—
A	86	2.84	74.0	61	3.95	96.9	83.5
B	79	2.87	77.1	93	2.61	65.2	70.7
C	66	3.12	83.4	51	3.35	79.6	81.7
D	64	3.31	92.8	42	4.69	117.5	102.6
E	60	3.65	93.2	58	2.90	81.4	87.4
Check	300	—	98.8	—	—	—	—
Significant difference between any pair at 5 per cent level							6.03

Examination of this table reveals a very marked negative relationship between the number of roots harvested and the mean weights or volumes such that it is evident that root competition is an important factor in bringing about variations in yield.

TABLE 7.—ANALYSIS OF VARIANCE OF VOLUMES OF SWEDES PRODUCED BY SEEDS OF DIFFERENT SIZES, 1946

Source of variation	Degrees of freedom	Sum of squares	Mean square
Between means of ten plots	9	1931.58	—
Between means of blocks	1	40.49	40.49
Between means of seed sizes	4	1218.01	304.50
Interplot error	4	673.08	168.27
Intraplot error	650	—	29.43

The interplot mean square is large compared to intraplot error; using the interplot mean square for estimating significance therefore,

$$F = \frac{304.50}{168.27} = 1.81$$

The 5 per cent point is 5.69, so that the effect of seed size is not significant. Such correlation as exists between seed size and root size is negative (-0.49) and not significant.

The check sample with 300 roots and mean volume of 98.8 compared with the bulk, of 125 roots and mean volume 78.6, and with the average of fractions A to E with a mean volume of 80.6, illustrates the variation in root size of seeds of the same variety of different origin.

Part II. 1947 Growing Season

MATERIALS

Two samples (Nos. 72 and 73) of Registered Laurentian Swede grown in the Maritimes were obtained for this study. They were separated into the same series of fractions as was the 1946 material, using a zinc screen with round holes, and each fraction was tested for germination in the Seed Research Laboratory. The following tabulation shows these particulars for the two samples used:

Lot	Size of screen retaining	Per cent by weight		Germination	
		Sample 72	Sample 73	Sample 72	Sample 73
A	1/14	26.5	31.2	92.5	96.0
B	1/15	34.9	37.7	91.0	96.5
C	1/16	20.5	17.6	90.5	97.0
D	1/18	17.1	12.5	88.5	96.5
E	1/22	1.0	1.0	90.0	91.0

There were no significant differences in the germination capacity except for Lot E of sample 73 which was just significantly lower than the other four lots of that sample. Sample 73, however, has a significantly higher germination than Sample 72.

METHODS

Detailed speed of germination trials were not made on these lots but a figure was obtained, indicative of germination speed, by the use of the formula

$$\frac{\text{Average germination at 4 and 7 days}}{\text{Final germination}} \times 100$$

Volumes were investigated in the same way as in the 1946 trials.

Field trials were conducted at Sackville, Macdonald College and Ontario Agricultural College. Unfortunately, at the two latter places, root maggot and weather, respectively, spoiled the plots. Some observations were possible at Macdonald College and these are referred to later.

At Sackville, plots for each sample were arranged in a Latin square, consisting of five sub-plots for each of the five seed sizes. Each sub-plot was 5 ft. \times 10 ft. and consisted of three rows. From the centre of each plot, 25 roots were harvested. Seeds were sown more thickly than needed and the seedlings thinned for spacing only, without regard to vigour, to 15 plants per row. If a plant died, another was transplanted to replace it in order to protect those on either side but this plant was not used when taking records; instead, an extra plant from the row was used. Appropriate guard rows were provided.

In spite of these precautions, three plots had so few roots that they were not acceptable for the analysis of the results. All three were from the smallest size fraction, E; one was in sample 72 and two in sample 73. Estimates of the yields of the missing plots were made according to the method outlined by Snedecor (8). Analysis of variance was carried out on the corrected data. Where an occasional root was missing at harvest time, the roots on either side of the space were not taken.

The plots were seeded on June 13 and harvested on October 17; the total weight of roots was recorded for each seed size, as were the polar and equatorial diameters of each root. Unfortunately, no records were taken of the distance of the plane of greatest width from the top or of other root characteristics such as pronginess, etc.

The weight-volume ratios were as follows:

Seed lot	Weight/volume ratios	
	Sample 72	Sample 73
A	0.0362	0.0369
B	0.0375	0.0369
C	0.0369	0.0370
D	0.0372	0.0378
E	0.0366	0.0350

These ratios are reasonably constant and do not differ much from those of the 1946 trials.

RESULTS

Speed of Germination

Table 8 gives the germination speed based on the laboratory germination tests.

TABLE 8.—GERMINATION SPEED—BLOTTER TESTS

Lot	Germination speed per cent	
	In sample	
	72	73
A	91.6	95.1
B	92.0	96.1
C	94.8	95.6
D	93.2	97.2
E	89.2	94.0

No differences in speed of germination which would be of significance in the present problem are evident although it is to be noted that sample 73, which showed somewhat greater germination capacity also shows the greater germination speed.

Volumes

The mean volumes for each seed size, after corrections for the missing plots are given in Table 9.

TABLE 9.—MEAN VOLUMES IN CUBIC INCH OF ROOTS OF LAURENTIAN SWEDES PRODUCED FROM SEEDS OF DIFFERENT SIZES

Lot	Sample 72	Sample 73
A	68.0	83.0
B	69.7	76.9
C	66.8	77.6
D	71.3	76.3
E	75.3	78.3
*	15.6	10.1

* Significant difference between any pair at 5 per cent level.

While there are rather few values for determining correlation between seed size and size of root, it is interesting to note that sample 72 gave a correlation of -0.875 and sample 73 one of $+0.47$, one negative and one positive. Neither correlation is significant, however.

Analysis of variance is shown in Table 10.

TABLE 10.—ANALYSIS OF VARIANCE OF VOLUMES OF SWEDES PRODUCED BY SEEDS OF DIFFERENT SIZES

Source of variation	Degrees of freedom	Sum of squares	Mean square
<i>Sample 72</i>			
Total	23	2872.54	—
Rows	4	1099.16	274.79
Columns	4	175.16	43.79
Seed sizes	4	218.22	54.56
Interplot error	11	1380.01	125.46
Intraplot error	574	—	19.636
<i>Sample 73</i>			
Total	22	1686.14	—
Rows	4	152.69	38.17
Columns	4	767.38	191.85
Seed sizes	4	248.30	62.08
Interplot error	10	517.77	51.78
Intraplot error	550	—	26.15

For both samples the intraplot error is significantly less than the interplot error. Using the latter, therefore, for testing significance, it is seen to be either greater than (72) or about the same magnitude as (73) the seed size mean square. It must be concluded, therefore, that seed size was not influential in determining root size.

Part III. Type Study and Uniformity in Size of Roots

RESULTS

Type

In order to investigate the relationship between size of seed and shape of the resulting root, an index of shape was calculated for each root. This index was the ratio:

$$\frac{\text{Difference between polar and equatorial diameters}}{\text{Average of polar and equatorial diameters}}$$

The index is positive for long roots, zero for "round" roots and negative for a flattened root.

Type, in the Laurentian variety of swede, varies considerably from year to year and appears to be profoundly affected by the combination of soil and weather conditions and a timing factor.* Accordingly, direct comparisons cannot be made between the 1946 and 1947 plantings. However, results in any one trial should be reasonably comparative. Table 11 presents the mean shape indices given by seeds of different mean sizes and Table 12, the analyses of variance of the data.

TABLE 11.—MEAN SHAPE INDICES GIVEN BY SEEDS OF DIFFERENT MEAN SIZES

Size of screen retaining	Lot	Sample		
		1946	1947	
			72	73
1/14	A	0.060	0.145	0.109
1/15	B	0.057	0.142	0.114
1/16	C	0.058	0.144	0.124
1/18	D	0.029	0.129	0.094
1/22	E	0.021	0.095	0.033
*		0.124	0.044	0.056

* Significant difference between any pair at 5 per cent level.

The bulk sample in the 1946 trials gave a mean index value of + 0.0978 and the "check" sample, of 300 roots, gave a mean index value - 0.0133, values which differ highly significantly. This illustrates the variability in this factor between different stocks of the same variety.

The analysis of variance of the 1946 results gives an interplot variance much greater than the intraplot error so that great heterogeneity among the plots was evident. The interplot variance is, in fact, slightly greater than that ascribable to seed size.

In samples 72 and 73, soil heterogeneity is also evident if in lesser degree. In sample 73 the inter- and intra-plot variances differ significantly, though not in sample 72. However, as the interplot error is the limiting factor in the precision of the experiments, it should preferably be used in testing significance. This gives $F = 3.37$ for sample 72, the 5 per cent and 1 per cent points being 3.36 and 5.67, respectively and $F = 4.60$ for

* Communication from L. C. Raymond, Professor of Agronomy, Macdonald College, Quebec.

TABLE 12.—ANALYSIS OF VARIANCE OF SHAPE INDICES OF SWEDES PRODUCED BY SEEDS OF DIFFERENT MEAN SIZES

Source of variation	Degrees of freedom	Sum of squares	Mean square
1946			
Between means of 10 plots	9	0.039121	—
Between means of blocks	1	0.003648	0.003648
Between means of seed sizes	4	0.015449	0.003862
Interplot error	4	0.020024	0.005006
Intraplot error	650	—	0.000336
1947—Sample 72			
Total	23	0.037819	—
Rows	4	0.009989	0.002497
Columns	4	0.003361	0.000840
Seed sizes	4	0.013467	0.003367
Interplot error	11	0.011002	0.001000
Intraplot error	574	—	0.000620
1947—Sample 73			
Total	22	0.050096	—
Rows	4	0.002097	0.000524
Columns	4	0.002498	0.000625
Seed sizes	4	0.029469	0.007367
Interplot error	10	0.016032	0.001603
Intraplot error	550	—	0.000678

sample 73, the 5 per cent and 1 per cent points being 3.48 and 5.99, respectively. Thus the effect of seed size on type of root produced may be considered significant.

The magnitude of these effects is illustrated in Table 11, which shows a definite tendency for the two smallest seed groups to produce flatter roots. In the 1946 results the design of the trial was such that the significance of this tendency could not be established. In sample 72 it will be noted that lot E differed significantly from lots A, B and C but not from lot D, whereas in sample 73, lot E differed significantly from all other lots.

Uniformity in Size

As a measure of uniformity in the sizes of roots produced by the different seed-size groups, the coefficients of variability have been calculated

$\frac{\text{S. D.}}{\text{Mean}}$ and are given in Table 13.

TABLE 13.—COEFFICIENTS OF VARIABILITY OF ROOT SIZES PRODUCED BY THE DIFFERENT SEED SIZE GROUPS

Year Lot	1946	1947	
		No. 72	No. 73
A	0.58	0.31	0.34
B	0.48	0.35	0.33
C	0.52	0.31	0.32
D	0.45	0.33	0.34
E	0.54	0.42	0.32

The coefficients clearly show no correlation with seed-size groups.

Thus, it may be concluded that size of seed has no effect either directly, as a result of differences in mean size (as shown in Parts I and II) or indirectly, as a result of lack of uniformity within the seed-size group, on the uniformity in size of roots in the whole field.

DISCUSSION

The qualities of promptness in germination, uniformity in size and uniformity in shape are all of importance to the grower of turnips. These qualities have been studied using registered seed of the Laurentian variety, screened to provide five fractions of different mean size, during the growing seasons of 1946 and 1947, in order to determine whether size of seed is a significant factor influencing these qualities.

No significant differences were found in laboratory determinations of germination speed of, or in the field, of mean size of roots produced from, seeds of different mean size, nor was there any difference in uniformity of root size among the different seed-size groups. The smallest seed group, however, produced significantly flatter shaped roots in the 1947 trials, but in the 1946 trials, the effect, while apparent, could not be stated to be significant.

The failure of the smallest size of seed to produce a satisfactory stand in the 1947 trials at Sackville, which was also experienced in the Macdonald College trials,* in spite of a high laboratory germination is also a factor which must be considered. This, with the fact of significantly different root shape suggests that material which goes through a 1/18 screen should be removed from seed stock. The removal of this fraction would involve a loss of about 1-1½ per cent by weight, if the samples used in this investigation are typical of Laurentian stock; a loss that might be considered negligible. Since these seeds are very small compared to seeds of the larger fractions, their retention would have a disproportionately great effect on field stand and root type, 1-1½ per cent by weight corresponding to 2-3 per cent by number. Of course, this is a very small proportion of the plants in the field and at thinning time, since this fraction is harder to establish, it is likely that it would be represented in still smaller proportion. However, the point chosen (seed passing through a 1/18 screen) may not be the most suitable and it is possible that, for example, seed passing through a 1/17 screen should be removed.

Further study would be needed to determine how uniform the Laurentian variety is in size of seed and in the proportions of seeds of different mean sizes when grown in different years and in different places, and whether it would be safe to make a sweeping statement that all seed smaller than a certain size should be removed. It is believed, however, that it is safe to recommend the removal of all seed that goes through a 1/18 screen since such seed may be considered abnormally small for any swede stock.

* Communication from L. C. Raymond.

SUMMARY

Seed of swede turnips of the Laurentian variety was screened into five fractions of different mean sizes which were tested in the laboratory for germination speed and germination capacity and in the field for yield, shape and uniformity of roots. Seeds of small size were in general as prompt to germinate and of as high germination capacity as seeds of large size, but failed to give as good a stand in the field in some plots. Roots produced by the smallest size of seed tended to be flatter in shape than those produced by the larger sizes, but did not differ significantly from the latter in mean size nor in uniformity of size. Owing to the difference in shape of the roots produced by seeds of the smaller sizes and their failure to become satisfactorily established in the field, it is recommended that the smallest size group be removed from seed stock.

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A STUDY OF NUTRITIONAL ANEMIA IN SUCKLING PIGS

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Since the demonstration by McGowan and Crichton in 1923 (11) that anemia in suckling pigs is due to a deficiency of iron in milk, practicable methods of treatment have been introduced which have greatly reduced, but have by no means eliminated, losses attributable to this disease. Undoubtedly further progress toward the elimination of swine losses from this source can be achieved by continued instruction of swine producers in the use of currently recommended preventive treatments, but recent evidence raises the possibility that these recommendations may not be adequate in all cases.

Apart from the discovery of the importance of copper in hemoglobin formation by Hart and co-workers (6), anti-anemia value under various conditions and in diverse species, has been attributed to numerous compounds including glutamic acid, arginine, lysine, pantothenic acid, pyridoxine, ascorbic acid, niacin and folic acid. Of these, only pyridoxine and folic acid have been associated with swine anemia. Hughes and Squibb (9), Wintrobe *et al.* (19) and Cartwright, Wintrobe and Humphreys (2) demonstrated the erythropoietic action of pyridoxine in pigs maintained on an artificial ration, while Cunha and associates found that folic acid stimulated hemoglobin formation to some extent in growing pigs receiving a purified diet (3, 4). In addition, folic acid has been shown to be essential in the formation of hemoglobin in the chick (8) and to be of value in the treatment of some cases of pernicious and nutritional macrocytic anemia in humans (17). That this vitamin might have some importance in the treatment of the anemias of suckling animals is suggested by the knowledge that cow's milk is a poor source of folic acid (1). However, Johnson, James and Krider (10) raised pigs from birth to eight weeks of age on "synthetic milk" lacking in folic acid, without producing consistent deficiency symptoms even when a bacteriostatic agent was employed.

Some of the data in the present paper were obtained from experiments conducted to study the influence of age at the time of initial dosage on the efficacy of the reduced iron method of treating anemia in suckling pigs. Blood hemoglobin levels are also reported for pigs maintained under practical conditions and treated with reduced iron plus either copper, pyridoxine, folic acid, or pyridoxine and folic acid. Limited data are included on hemoglobin levels recorded for suckling pigs allowed access to pasture.

EXPERIMENTAL

Investigations were conducted at the University of Alberta from 1946 to 1948 to obtain information concerning the comparative value of several treatments for nutritional anemia in suckling pigs. All pigs were farrowed by sows receiving a gestation-lactation ration of equal parts oats and barley, with pasture in season, and either 6 per cent or 12 per cent mixed protein-mineral supplement depending upon the availability of pasture.

Unless otherwise designated, all litters were retained indoors in pens with concrete floors and wooden sleeping platforms during the course of the experiment.

Treatments

I. Reduced iron

Hart (7) has denoted a level of 175 mg. of iron per week as sufficient to meet the needs of suckling pigs and this figure has been widely accepted. Reduced iron, which was introduced as a practical treatment for swine anemia by Schofield (14), was administered in these experiments at the rate of 210 mg. per week in a single dose. In order to increase the accuracy of administration, the reduced iron was mixed with dextrose to provide 210 mg. of reduced iron per gram of mixture. One gram of this mixture was weighed into a small envelope from which it was deposited on the back of the pig's tongue. The importance of age at the time of initial treatment was studied by beginning weekly therapy at the following ages: (a) 1 day (b) 3 days (c) 4-6 days (d) 8 days. Littermate controls were maintained in all groups except those receiving treatment (c).

II. Reduced iron plus copper sulphate

Copper sulphate was added to the reduced iron and dextrose mixture described under Treatment I at a rate calculated to provide 35 mg. copper per week (7). The pigs were treated weekly beginning at one day of age and littermate checks were maintained on reduced iron alone.

III. Vitamin treatments

- (a) Reduced iron plus 10 mg. folic acid per week.
- (b) Reduced iron plus 25 mg. folic acid per week.
- (c) Reduced iron plus 30 mg. pyridoxine per week.
- (d) Reduced iron plus 10 mg. folic acid plus 30 mg. pyridoxine per week.

The vitamins were administered five times per week in powder form with a dextrose base beginning at 4-6 days of age. Iron was given at weekly intervals to the test pigs and their littermate controls beginning at the first day of vitamin supplementation.

IV. Pasture

Limited observations were made on the value of giving pigs access to pasture at 11 days of age. Others were turned on pasture at 22 days of age after having been treated with iron or iron and copper.

Blood Samples

Blood obtained from a marginal ear vein was measured in a 20 cu. mm. hemoglobin pipette. The hemoglobin values were determined by the method of Evelyn (5) using an Evelyn photoelectric colorimeter.

RESULTS AND DISCUSSION

Iron Treatments

The hemoglobin values obtained for the iron-treated pigs are shown graphically in Figure 1.

From the results shown in Figure 1 it is apparent that the age at which the iron treatment was begun was an important factor in determining the degree of success attained in combating anemia in these suckling pigs.

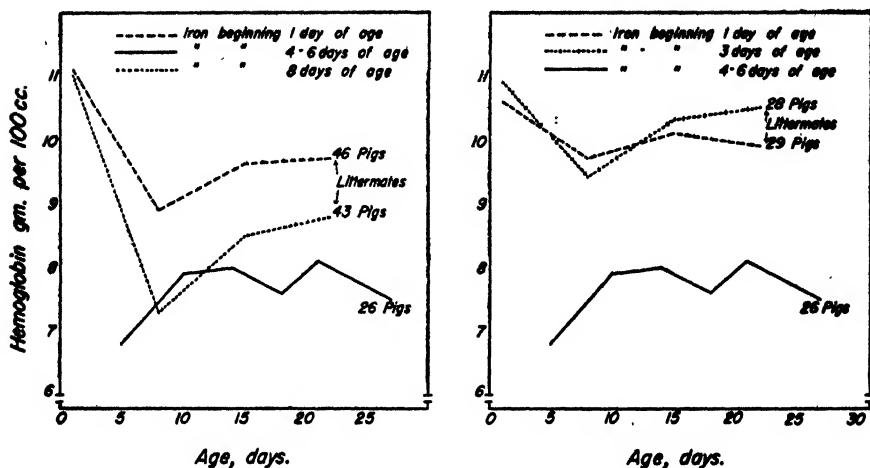


FIGURE 1. The importance of age at the time of initial treatment for anemia.

The average values obtained for the pigs treated initially at 4-6 days of age represent a condition of moderate anemia, whereas those for the animals dosed at one or three days show a satisfactory hemoglobin level. Several of the pigs in the group treated at 4-6 days remained acutely anemic, even after reduced iron therapy. The hemoglobin picture for those treated at 8 days resembles that of the pigs dosed at 4-6 days, but appears to be slightly more favorable at about three weeks of age. This difference may be attributable to inter-litter variation which is known to be extensive (15).

It has long been recognized that blood hemoglobin may fall to a dangerous level within a few days after birth, and mainly for this reason a number of investigators (14, 18, 16, 13) have recommended early treatment. It would appear, however, that this aspect of swine anemia control has not been sufficiently emphasized, since a number of current recommendations still call for treatment beginning at one week of age. The results summarized in Figure 1 show that when therapy is postponed beyond the third day, there is not only a rapid initial decline in hemoglobin levels, but in addition the level attained after treatment may, for a period of at least two to three weeks, remain well below that of pigs treated within 3 days of birth.

Iron and Copper Treatment

The hemoglobin values of 31 twenty-two-day-old pigs treated with reduced iron and copper sulphate averaged 10.8 gm. per 100 cc. of blood while those of 36 littermates receiving only reduced iron averaged 10.7 gm., indicating that the pigs were not deficient in copper.

Vitamin Treatments

From the results summarized in Figure 2 it is obvious that, at the levels employed in these experiments, neither folic acid nor pyridoxine, either singly or in combination, was effective in increasing the hemoglobin content of the blood of suckling pigs treated with reduced iron. Results obtained with six pigs dosed with folic acid at the rate of 25 mg. per week suggest that at this high level folic acid may have a depressing effect on the rate of hemoglobin formation, particularly during the first three or four weeks.

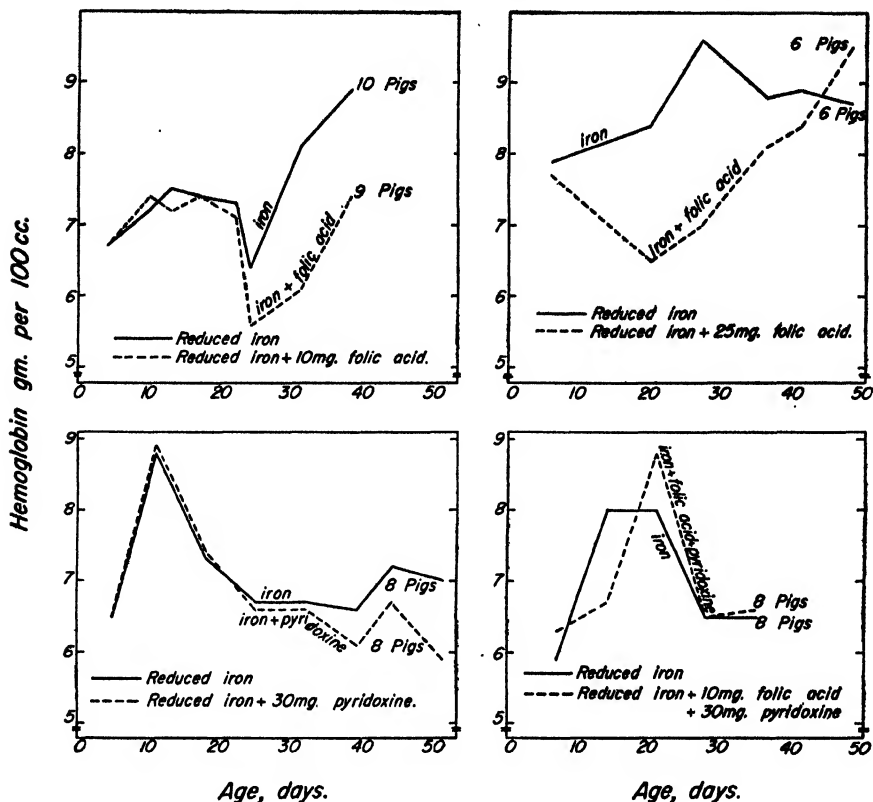


FIGURE 2. The effect of treating littermate pigs with reduced iron vs. reduced iron plus folic acid* and/or pyridoxine on the hemoglobin content of the blood. Vitamin levels indicated are on a weekly basis.

*Folic acid was supplied by Lederle Laboratories, Pearl River, N.Y., through the courtesy of E. L. R. Stokstad.

Pasture Treatment

Litter 201Z was placed on pasture at 11 days of age with no previous treatment for anemia. As shown in Table 1, a spontaneous regeneration of hemoglobin occurred in all pigs.

TABLE 1.—THE EFFECT ON THE HEMOGLOBIN LEVEL OF GIVING PIGS ACCESS TO PASTURE

Pig No.	Age, days		
	11	14	17
1	5.4	7.8	10.2
2	6.3	7.8	9.3
3	6.3	8.1	9.2
4	6.8	8.3	9.2
5	5.3	8.4	9.6
6	5.1	8.0	9.0
7	5.4	7.9	9.8
8	5.0	7.9	8.3
Average	5.7	8.0	9.3

The satisfactory response obtained from giving young pigs access to pasture suggests that this may be the safest means of protecting them from anemia. The resulting increase of 63 per cent in the hemoglobin level over six days is in sharp contrast to the increases of 18 per cent and 16 per cent obtained over a comparable period following iron treatment at 4-6 or 8 days of age respectively (see Figure 1). One hundred and thirty-eight 22-day-old pigs having a previous history of iron or iron and copper treatment, which were turned on pasture with an average hemoglobin reading of 10.02 gm., underwent a mean increase of .63 gm. or 6.3 per cent in four days.

Hemoglobin Levels at Birth

An average hemoglobin value of 10.84 gm. was found among 208 pigs tested within 24 hours of birth. These were comprised of equal numbers of males and females, the mean for the males being 10.70 gm. and for the females 10.97 gm. With respect to the observation of Mitchell (12) that suckling female rats possess a greater resistance to anemia than males, it is interesting to note that no such difference was found in the present study with pigs.

SUMMARY AND CONCLUSIONS

Experiments are described in which it was observed that the hemoglobin content of the blood of suckling pigs treated with reduced iron at weekly intervals beginning at 1 to 3 days of age, remained appreciably higher over a period of three weeks than that of comparable pigs treated initially at either 4 to 6 or 8 days of age. These results emphasize the importance of initiating iron treatment on or before the third day following birth. Pigs treated with commercial reduced iron did not show any additional response from supplementary copper. Likewise, hematopoiesis was not accelerated by additions of folic acid or pyridoxine. The rate of hemoglobin regeneration in anemic pigs given access to pasture was significantly greater than in those treated with reduced iron.

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MINOR ELEMENT DEFICIENCIES AFFECTING CANADIAN CROP PRODUCTION¹

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An increase in the prevalence of minor element deficiencies may be expected as the pressure of increased population compels more extensive land use and perhaps the use of poorer sub-marginal soils. To attain higher production greater use is being made of commercial fertilizer and while this may be a desirable practice it results in a heavier drain upon nutrients not supplied in the fertilizers. Unless especial attention is paid to soil management higher production may result in humus depletion and humus is an appreciable source of the minor elements essential for plant growth. While the more obvious occurrences of minor element deficiencies in Canada have been diagnosed and fairly satisfactory control measures established, there may be a much more extensive decrease in production arising from such factors, unsuspected because not associated with marked abnormal plant growth symptoms.

BORON

Apples

Working independently, McLarty (30) in British Columbia, Hill and Davis (15) in Ontario and Quebec, and Young (38) in New Brunswick associated physiological disorders of the apple known as drought spot, internal cork and corky core with a deficiency of boron. Although this association was not determined until 1936 these disorders had been recorded in various fruit-growing areas for a number of years, increasing in severity and economic importance from 1921 to 1935. McLarty and Wilcox (31) recommended broadcasting boric acid crystals at the rate of eight ounces per tree over a circular area at least twenty feet in diameter around each tree to be treated. Hill and Davis (15) showed that, common with other boron deficiency diseases, these disorders are influenced by high lime and nitrogen content of the soil, and Davis (7) later gave control recommendations as follows: "Cork disorders may be corrected by boron application to the soil at the rate of four to eight ounces of borax per tree, applied in spring and worked into the soil if possible. If the soil is alkaline or on the high lime side immediate results may be obtained by incorporating borax with the regular lime sulphur sprays. If this is done two applications should be sufficient, one at the time of the calyx spray and the other, the second spray after that using borax at the rate of 2½ pounds to 100 gallons of spray mixture." In 1937 Middleton and McLarty (36) advocated treatment of all orchard land in the Okanagan and Kootenay valleys of British Columbia with boric acid at the rate of thirty pounds per acre. Apple trees which have not been affected should be treated as a preventive measure; but trees which received boron during the previous season should not be treated again for some years. In 1939 traces of corky core and drought spot were found on a few trees treated with one-half pound boric acid in 1936, and McLarty (32) accordingly recommended that a second application

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be made in the autumn of 1939 to orchards treated in 1936. Up until 1940 about two-thirds (15,000 acres) of the British Columbia orchard land had been treated. McLarty also recognized five symptomatic diseases of apples due to boron deficiency, namely, corky core, drought spot, flat fruit, measles and die back. Eaves and Hill (18) also reported that a single treatment, applied as a soil treatment or a spray, proved effective for a period of three years.

While boron deficiency in apples had not been so prevalent in Nova Scotia (23) as in other areas it occurred fairly frequently in individual trees or small groups of trees and recommendations have been made for its use as a preventive measure.

It will be seen that boron deficiency of apples is of major economic importance in all the main fruit-growing areas.

In British Columbia the present control recommendations are to apply a soil application of boric acid every third year at the rate of thirty pounds per acre. In the provinces of Nova Scotia and New Brunswick use is made of a 9-5-7 fertilizer containing four per cent commercial borax to be employed every third or fourth year. In the province of Quebec boron is applied as a foliage spray adding $2\frac{1}{2}$ pounds of borax to 100 gallons of regular lime sulphur or mild sulphur spray mixture in the calyx and second cover spray. Recommendation for treatment is made through the spray service calendar every third year, and this serves as a convenient manner of maintaining the necessary boron supply without the possibility of creating an excess.

In Ontario many of the orchards previously affected with boron deficiency are located on alkaline soils. On such soils effective control may not always be obtained by soil application in a season when very low soil moisture conditions exist. For this reason greater use is made of the foliage spray treatment.

Other Fruits

It is only in the province of British Columbia that boron deficiency has been definitely identified on fruits other than the apple. In 1941 McLarty (33) stated: "In respect to the Okanagan district it is now well established that all agricultural land in this district is generally deficient in boron unless it already has received a treatment. Crops which have definitely benefited from such treatment are all orchard trees (apple, pear, peach, apricot, prune and cherry), raspberries, currants, turnips, mangels, beets, alfalfa, sweet clover, celery, spinach, lettuce, wheat and oats."

McLarty (34) also stated that boron deficiency on other fruits is less clearly defined than on the apple and that other factors may cause a condition in the fruit that is similar. The condition in pear fruit that has responded to boron treatment is described as follows: "The calyx end of the fruit is depressed; there is an excess of stone cell development and large cracks may develop on the surface midway between the calyx and stem-end. A roughening of bark tissue which corresponds to the 'measles' on apple twigs also occurs. The dying back of pear twigs in the spring, in the same manner as in the apple, is a reliable symptom of boron deficiency."

Drought spot is sometimes found on cherry and apricot and on plum some gum spotting occurs, but not all has been cured with boron. With the exception of the cherry, stone fruits are as susceptible to die back as

apple or pear. Fitzpatrick and Woodbridge (12) described definite foliage and die back symptoms of boron deficiency in apricots with plants grown in sand culture. Symptoms similar to those described from the plants grown in sand were found in several apricot orchards in the Okanagan Valley in the spring of 1940.

Turnips

The use of boron has become of major economic importance for the control of brown heart or water core in the production of the turnip. In New Brunswick about two-thirds of the 9300 acres in turnips were treated with borax in 1945. In 1947 the approximate tonnage of borax-containing fertilizers for the Maritime Provinces was 3353 tons and straight sales of borax were approximately 20 tons. Experiments in Prince Edward Island indicated that in a normal cropping plan, when turnips come only once in four or five years, they require borax each time they occur in the rotation (22). The Advisory Fertilizer Board for Ontario has also recommended that boron be employed for turnip production.

Turnip brown heart has been under investigation in Canada since 1928, a year when this disease proved to be the direct cause of very considerable losses to farmers in Eastern Canada. McLeod and Howitt (28) demonstrated that borax applied directly in the drill at the rate of ten pounds per acre effected a satisfactory control of this disorder. Hill and Grant (16) studied the symptoms of boron deficiency as exhibited by turnips growing in sand culture and their observations were confirmed and extended by Coulson and Raymond (6). Hurst and MacLeod (22) reported on further tests conducted during 1933-35; 15 to 20 pounds of borax per acre gave satisfactory control on a variety of soils and proven methods of applying the borax were as follows: (a) in the drill; (b) at the sides of the drill; (c) broadcast and (d) combined with the fertilizer. Satisfactory control was also obtained by spraying the required amount of borax on the soil or the foliage of the turnip.

On some nearly neutral soils in the province of Quebec as much as fifty pounds of borax per acre is required to give adequate control of brown heart, while on other slightly acid soils fifteen pounds per acre will suffice.

In New Brunswick, Bailey (1) found borax applications in the drill as low as fifteen pounds per acre and sprays from twenty to eighty pounds per acre gave effective control of brown heart if applied at any time before the roots began to swell. Recommendations for the provinces of Prince Edward Island, Nova Scotia and New Brunswick for 1949 are as follows: A special 3-15-6 or 3-12-10 fertilizer with three per cent commercial borax added; where lime has been applied, add borated mixtures or borax enough to supply fifteen to twenty pounds per acre.

In Ontario spraying or dusting for the prevention of water core is now being used generally (25). This is especially true in the high-lime soil areas where soil applications of borax have failed to give results. The recommended spray ingredients are twelve pounds of powdered borax, three pounds of bentonite clay and one pint of Orthex spreader in forty imperial gallons of water. Forty to fifty gallons of spray should be applied per acre when the diameter of the turnip root is between 1 and 1½ inches.

The recommended dust is finely powdered borax (300 mesh) and Celite (No. 209) mixed in equal proportions by weight.

Alfalfa

In the course of experiments with apples, McLarty (35) found that application of boric acid had a marked effect in improving the colour and growth of the alfalfa cover crop. Subsequently alfalfa yellows was found in a considerable number of fields in British Columbia and McLarty stated that land in alfalfa should receive an application of thirty pounds borax per acre where the alfalfa shows a yellow-top condition and fifteen pounds per acre where the plants remain green as a preventive measure. Although the disorder has been encountered in individual fields in other provinces its incidence has not been so common as to warrant a general recommendation of boron treatment for fields in alfalfa.

Celery

Ferguson and Wright (11) described and illustrated boron deficiency symptoms on celery when grown in sand culture. Browne (34) reported that on mildly acid peat soils celery tends to develop boron deficiency diseases in the third or fourth year of cropping. Occasional applications of fifteen or twenty pounds of borax per acre are therefore recommended on such soil type. In extreme cases quicker results can be obtained by dissolving two or three pounds borax per one hundred gallons in at least two of the celery sprays, applied when the crop is from one-third to one-half grown.

A general recommendation is given for celery growing on muck soils in Ontario and Quebec. On mineral soils it is advised that the need for boron be fully confirmed before application is made and a rate of fifteen to twenty pounds of borax per acre is suggested.

Carrots

In sand culture without boron, Hill (17) found carrots turned yellow around the edges of the leaflets, followed by a distinctive pinkish-to-reddish colouration spreading farther into the leaf. Roots of such plants usually developed longitudinal cracks or splits. Browne (45) found that on mildly acid peat soil longitudinal cracking of carrots was completely controlled by soil applications of twenty pounds of borax per acre when in the control plot 62 per cent of the roots were cracked. In British Columbia Harris (13) obtained a significant increase in yield of carrot roots by borax manuring at the rate of fifty pounds per acre though the crop without borax showed no sign of disease.

Other Crops

Boron deficiency of other crops has been of isolated rather than general occurrence.

In sand cultures with tomato, Hill (18) described both foliage and fruit symptoms due to lack of boron which was later confirmed by Ferguson and Wright (11). Ferguson and Wright (11) also produced and described boron deficiency symptoms in cauliflower, cabbage and corn when grown in sand culture, and by the same method Hill (17) described boron deficiency in beet, spinach, carrot and garden pea. Leggatt (24) has reported abnormal germination of pea seeds which appeared perfectly normal but which were grown in a boron-deficient soil area in British Columbia. The addition of boron completely overcame the special type of abnormal sprouts.

COPPER

Up until the present time in Canada, a need for and response to copper application has been established only for certain crops produced on peat or muck soils. Browne (5) states: "Copper is essential in the fertilizer application for onions, carrots, lettuce and spinach. It is probably necessary for potatoes, but since in normal spraying or dusting comparatively large quantities of copper sulphate are used a deficiency with this crop is not likely to occur. Onions appear to need more copper than the spray residue from a previous potato crop and also respond to copper on neutral or alkaline mucks. The application of copper on onions improves skin quality and increases yield. In general the application of 50 to 100 pounds of copper sulphate per acre is sufficient." The Ontario Fertilizer Advisory Board also recommends the use of copper for such crops on peat or muck soils.

COBALT

Soil cobalt deficiency appears to have a primary effect on the health of animals feeding on the crops thereon produced, rather than on the general vigour and growth of plants. In Canada widespread cases of animal malnutrition due to cobalt deficiency have not been established though a deficiency is suspected in certain areas.

Bowstead and Sackville, (2) of the University of Alberta, describe the value of various supplements in combating an unthrifty condition of sheep which developed when they were maintained on a basal diet of non-leguminous hay and ground oats. Certain ewes were given a cobalt supplement equivalent to five milligrams of cobalt daily. There followed a rapid increase in weight and improvement in thrift. Analyses indicated that the non-leguminous hay produced in the area was very low in cobalt. Subsequent studies confirmed these findings.

Soils from Cape Breton County, Nova Scotia, where a deficiency of cobalt was suspected, were analysed. The soils suspected of cobalt deficiency showed a total content of 2.7 p.p.m., while that representing an area considered to be satisfactory showed 8.9 p.p.m.

IRON

Iron deficiency encountered is of the lime-induced chlorosis type. It constitutes a serious problem on high carbonate alkaline soils of the three Prairie Provinces, Manitoba, Saskatchewan and Alberta, in the production of fruit, ornamental shrubs and shade trees. Control measures so far employed have been only partially satisfactory and temporary in nature. Direct injection of powdered iron salts, ferrous sulphate, ferric citrate, etc., have been proved more satisfactory than soil applications or foliage sprays. In 1947 a special committee was formed to study this problem. It was considered that soil amendments have application in certain restricted areas where the deficiency symptoms are not severe. It was felt that probably the most hopeful approach was through the question of varietal resistance. In the case of plants that are budded or grafted this might involve rootstock resistance and would involve a breeding programme with the purpose of breeding and propagating rootstocks definitely resistant to lime-induced iron deficiency.

MANGANESE

A deficiency or response to manganese has only been reported in isolated instances, occurring on calcareous, alkaline soils.

In the Horticultural Division of the Experimental Farms Service at Ottawa manganese deficiency has occurred on soya beans on an alkaline soil. Good control was obtained by spraying the foliage with one per cent manganese sulphate. Harris (14) in British Columbia has shown that, on a glaciated sandy loam upland soil, the addition of manganese significantly increased yield, total carbohydrates and vitamin C with the Cuthbert variety of raspberry.

Since 1923 certain areas at the Central Experimental Farm at Ottawa have been known as "sick soil areas" and oat plants grown on this area showed symptoms of grey speck. Timonin (37) has reported on soil studies in this connection. It was determined that a susceptible variety of oats harboured in its rhizosphere a denser population of manganese-oxidizing, casein hydrolyzing and denitrifying bacteria than the rhizosphere of a resistant variety. On application of soil fumigants such as chloropicrin, cyanogas and formaldehyde the bacteria capable of oxidizing manganese were reduced or eliminated. The plants grown in such treated soil were free from symptoms of manganese deficiency. The application of 190 pounds per acre of commercial calcium cyanamid resulted in practical control.

McLachlan (26) showed that chlorotic oats growing on a localized area at the Ontario Agricultural College, Guelph, were caused by manganese deficiency and that the condition was remedied by spraying with a one per cent solution of manganese sulphate with bentonite and soap added as a sticker and spreader; soil applications however, gave only minor response. The cause was apparently biological in nature since bacteria were isolated which actively converted $MnSO_4$ to the oxide form in vitro. He later (27) noted that the addition of sulphur at one thousand pounds per acre increased the yield and in one instance reduced the amount of manganese necessary as a soil application by about two-thirds. The application of a two per cent spray of manganese sulphate with a sticker spreader produced good control. Browne (4, 5) has shown that on a muck soil the quality of celery can be improved by applications of manganese sulphate up to fifty pounds per acre.

ZINC

Zinc deficiency has not yet been observed or identified in Canada. It occurs in the State of Washington, bordering British Columbia, and it is strongly suspected that it exists in some parts of the Southern Okanagan in British Columbia.

MOLYBDENUM

Molybdenum deficiency has not yet been observed or diagnosed in Canada.

MAGNESIUM

Although magnesium may not be considered as a minor element it is not generally directly applied as a regular fertilizer.

Its importance in quality tobacco production in Canada, however, has been fully recognized. Horton (21) reported the occurrence of sand drown in the New Belt and associated it with low available magnesium content of the soil. McEvoy (29) has published the optimum nutrient requirements for tobacco. Fertilizer recommendations have taken this need into account and are as follows in respect to magnesium: Owing to the frequent occurrence of magnesium deficiency or "sand drown" it is recommended that a high grade dolomitic limestone, containing at least 19 per cent MgO, be used as a filler in all tobacco fertilizers. For cigar tobacco it is noted that the addition of 2 per cent MgO to the fertilizer mixture tends to improve the quality of cigar tobacco.

Magnesium deficiency is of considerable importance in potato production in the Maritime Provinces. Magnesium deficiency in New Brunswick was first noticed in 1924 and gradually increased until in 1933 it had become a limiting factor in potato production (9). Co-operative experiments conducted by the Fredericton Laboratory of Plant Pathology and the New Brunswick Department of Agriculture showed that the application to the soil of 75 to 150 pounds of dolomitic lime or 60 to 120 pounds of magnesium sulphate per acre corrected the trouble. Spraying the growing crop with similar amounts of magnesium sulphate in solution also cured this disease. Magnesium deficiency was very prevalent in Prince Edward Island in 1943, being due to one or more of the following factors: high soil acidity; heavy applications of acid-forming fertilizers containing no magnesium; low organic matter content of the soil. Fields that received an application of Bordeaux mixture containing ten pounds of magnesium sulphate in eighty gallons showed a marked improvement and three applications completely cured the trouble. Increased yields were also obtained by applying thirty pounds per acre of magnesium oxide mixed with the 4-8-10 fertilizer.

After subsequent experiments the Maritime Fertilizer Council have recommended that for potatoes on very acid soils a fertilizer mixture such as a 5-10-13, 5-10-10 or 6-9-12 with one per cent added MgO should be employed.

In 1938 Hill (19) described and diagnosed magnesium deficiency occurring in severe form in an apple orchard area in the province of Quebec. Since that time it has occurred in this district in individual orchards with varying severity from year to year and has also occurred in individual orchards in Ontario and Nova Scotia. Hill and Johnston (20) later described magnesium deficiency symptoms of the apple as occurring in sand culture and in commercial orchards. Field experiments have shown that effective response to surface broadcast applications of magnesium sulphate on bearing sod orchards may be delayed for a period of two to three years and the content in the foliage is likely to drop below requirements within a subsequent period of two years. Three to four cover sprays of two per cent magnesium sulphate were effective in correcting magnesium deficiency in the year of application. This treatment is recommended as a quick, temporary control measure. Although the magnesium content of the foliage was satisfactorily increased in the same season of treatment the effect was sometimes not carried over even to the next growing season. Magnesium deficiency affecting the apple has been generally encountered on strongly acid soils. It was found that an application of 50 to 75 pounds of dolomitic

limestone spread around each tree effectively increased the magnesium content of the foliage the third year after application. It is recommended that an application of dolomitic limestone be made on such soils and that during that period before the limestone application is effective, immediate but temporary control be obtained by foliage sprays.

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RESULTS OF STUDIES OF CRESTED WHEATGRASS¹

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INTRODUCTION

The Dominion Range Experiment Station, Manyberries, Alberta, is located in an area which is representative of nearly 8,000,000 acres of land unsuitable for production of annual crops. Lack of moisture and the high rate of evaporation are the principal factors limiting growth throughout the area. Attempts to produce wheat, oats, and other annual cereals have been generally unsuccessful. It is recognized now that a type of agriculture, based on the utilization of native and cultivated grasses, is the best land use practice for the region.

The Manyberries Station was established in 1927 to investigate the problems of land and grass use in the dry section of the Prairie Provinces. One of the most important problems at that time, and equally so at present, was to maintain the grazing capacity. Experiments were started to investigate the relationship between this factor, grazing practices, range management, water development, feed reserves, land use, and the utilization of native and cultivated grasses. Many of the results have been recorded in Results of Experiments, 1927-1936, inclusive (5). This paper deals in a general way with the results of crested wheatgrass reseeding experiments on abandoned farm land and depleted native pasture.

PHYSICAL FEATURES

The greater part of the region represented by the Station is from flat to gently rolling. In places the prairie is deeply cut by "coulees", steep-banked eroded water courses which are dry most of the year. Where erosion has been most severe, "badlands" have formed. These areas are sparsely, if at all, vegetated due to the constant action by wind and water. Drainage is largely into sloughs, although the local drainage basin is a part of the Milk River system, which in turn flows into the Missouri River.

The soils of the area are mainly light loams. The profile is associated with the residual rock formations which, in this case, are principally Bearpaw and Belly River shales. Practically all of the area has a solonized profile, with characteristic eroded pits due to the patchy removal of the "A" or surface horizon. These eroded pits may or may not support vegetation. The exposed "B₁" horizon is usually dark in colour and very hard, thus preventing or limiting water penetration into the subsoil.

Table 1 shows the fertility of the soil in the Manyberries district.

The climate of the area is marked by low precipitation, a high rate of evaporation, great extremes of temperature, frequent high winds, and abundant sunshine. In Table 2 a summary of the climatic information for the Station is presented.

¹ Contribution from the Division of Forage Plants, Experimental Farms Service, Dominion Department of Agriculture.

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TABLE 1.—SOIL NUTRIENTS FOUND IN THE LIGHT LOAMS OF THE DOMINION RANGE EXPERIMENT STATION, MANYBERRIES, ALBERTA

Depth	Horizon	Total N.	Total P.	Calcium	Magnesium	pH	P. p.p.m.
0 - 5"	A ₁	0.108	0.035	0.39	0.25	6.1	17
5 - 7"	A ₂	0.060	0.027	0.44	0.28	7.1	12
7 - 12"	B ₁	0.111	0.290	0.48	0.82	8.3	25
12 - 18"	B ₂	0.086	0.037	1.29	0.85	8.7	78
At 20"	C	0.066	0.059	0.50	0.86	8.4	44

(Courtesy, Survey of Milk River Sheet, by F. A. Wyatt, J. D. Newton *et al.*, University of Alberta.)

It will be noted in the table that the total annual precipitation is only 11.31 inches, and the precipitation : evaporation ratio is 0.27. This factor is the principal climatic condition limiting plant growth. While an average annual snowfall of 34.12 inches may appear high, it does not remain throughout the winter because of the many warm "chinook" winds which melt the snow, often as soon as it falls.

The vegetational cover is classified as short-grass prairie (1), and is characterized by the dominance of blue grama grass, *Bouteloua gracilis* (H.B.K.) Lag., which comprises over one-third of the total vegetative cover, and common spear grass, *Stipa comata* Trin. and Rupr. Other important grasses are western wheat grass, *Agropyron Smithii* Rydb., Junegrass, *Koeleria cristata* (L.) Pers., and dwarf bluegrass, *Poa secunda* Presl. Involute leaved sedge, *Carex eleocharis* Bailey is abundant, while nigger wool, *Carex filifolia* Nutt. is of frequent occurrence. Common broad leaved plants include pasture sage, *Artemisia frigida* Willd., dwarf phlox, *Phlox Hoodii* Richards, broom weed, *Gutierrezia diversifolia* Greene, winter fat, *Eurotia lanata* (Pursh.) Moq., salt sage, *Atriplex Nuttallii* S. Wat., and hoary sage bush, *Artemisia cana* Pursh., cactus, *Opuntia polyacantha* Haw., is very common at local points. Little club moss, *Selaginella densa* Rydb., is very abundant over all of the area. Its effect on the native vegetation is still a debatable question. Some authorities credit it with little or no influence on the vegetation, while others feel it stunts the growth of surrounding plants. In general, the vegetation is highly nutritive and palatable, but is of low productivity. The combined cover of all grass species is usually less than 10 per cent, but forbs and weeds increase the total vegetational cover from 20 to 35 per cent.

ECOLOGY OF CRESTED WHEATGRASS

The grass most highly recommended for reseeding in the area represented by the Manyberries Station is crested wheatgrass, *Agropyron cristatum* Gaertn. This grass, a native of the Steppe region of European Russia and Southwestern Siberia, was introduced into the United States in 1898. It did not attract much attention until after 1915 when its adaptability to cool, dry areas was observed. About this time the University of Saskatchewan obtained a small amount of seed. Small experimental plots were started and were the beginning of a crested wheatgrass seed industry that was the source of seed used to sow abandoned farm lands after 1935 in Western Canada.

TABLE 2.—METEOROLOGICAL RECORDS—DOMINION RANGE EXPERIMENT STATION, MANYBERRIES, ALBERTA
1929-1947 (19 years)

Month	Temperature F.			Precipitation			Bright sun (hours)	Wind velocity (hourly) 12 year av.	Evaporation (in.)
	Highest	Lowest	Mean	Rain (in.)	Snow (in.)	Total precip. (in.)			
January	61	-44	11.7	0.00	5.56	0.56	99.7	11.3	—
February	67	-45	13.5	0.00	4.15	0.42	122.8	12.0	—
March	73	-27	26.0	0.10	6.39	0.74	145.2	11.8	—
April	90	-15	42.4	0.63	2.91	0.92	195.6	12.8	—
May	94	12	52.7	1.23	0.08	1.24	251.7	12.1	5.54
June	105	30	59.7	2.44	0.00	2.44	247.6	10.9	6.42
July	103	34	69.0	1.31	0.00	1.31	341.0	9.2	8.31
August	104	17	65.8	0.75	0.00	0.75	300.7	10.0	7.01
September	96	10	54.4	1.01	1.31	1.14	194.5	10.3	4.33
October	91	-11	44.2	0.40	2.59	0.66	163.0	10.9	—
November	70	-26	26.7	0.02	5.72	0.59	110.4	10.7	—
December	63	-32	17.9	0.00	5.41	0.54	92.6	11.3	—
Annual	—	—	40.3	7.89	34.12	11.31	2,264.8	11.1	31.61

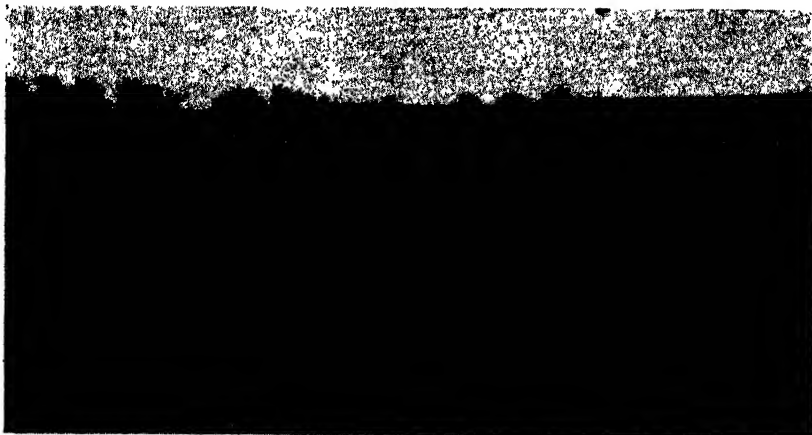


FIGURE 1. Site 2—Sheep grazing on crested wheatgrass sown during April, 1932.

Crested wheatgrass is a perennial. Its longevity is well illustrated by the seedlings made at the Northern Great Plains Field Station, Mandan, N.D., in 1915. As Westover and Rogler (1947) pointed out, the fifth highest yield over the 33-year period of growth was in 1942, the twenty-seventh year of production. The highest yield was in 1916, production being 3,550 pounds of hay per acre. The lowest, 146 pounds, was produced during the severe drought year of 1936. The average yield for 30 consecutive years has been 1,675 pounds. This is similar to the findings of the North Montana Branch Experiment Station, Havre, Montana, and at the University of Saskatchewan (2). From the evidence of these old stands, it seems feasible that most stands would yield well for 20 to 25 years. What is more important is the fact that even the poorest yields are far greater than those of the native vegetation in a corresponding year. The yields quoted from Havre, Saskatchewan, and Mandan are much higher than those obtained at the Manyberries Station.

Crested wheatgrass is well adapted to an extreme climate. During the 30 years it has been growing at the University of Saskatchewan, and the 20 years at the Manyberries Station, there has never been evidence of any frost damage or winterkilling, even with temperatures as low as 40° to 50° F. below zero.

Crested wheatgrass is drought tolerant; it endures long periods of drought without permanent damage. This ability is credited in part to its very extensive root system, which is able to get moisture from the soil when other less hardy grasses would perish (3). During long periods of summer drought, it becomes dormant and thus is able to avoid serious drought injury. As soon as moisture is available, the grass begins to grow and produce nutritious forage.

Crested wheatgrass is an excellent weed competitor. This is well illustrated by old experiments carried out at the Station, where seedings were made on land covered with annual weeds. Even when the grass was planted in 3-foot rows, the weeds were completely controlled within a few years of planting.

EXPERIMENTAL

Reseeding on Abandoned Dryland

As reseeded represents an important part in any range management program, several varieties of grasses and legumes were obtained and seeded as part of the Range Management Program at the Manyberries Station. Reseeding experiments were established on both native sod and on abandoned farm land in various stages of regression. Different rates of seeding and different cultural treatments were used. The first observations made of these seedings recorded the superiority of crested wheatgrass over all other species. By the time the several seedings were studied in 1948, only crested wheatgrass persisted of the several species seeded, and thus is the only species discussed in this article.

Site 1 is an abandoned field located approximately four miles southwest of the Station. It is a 70-acre field that had been abandoned prior to 1928, and had grown up to weeds, consisting of Russian thistle, *Salsola pestifer* A. Nels., pasture sage, *Artemisia frigida* Willd., tumbling mustard, *Sisymbrium altissimum* L., wild barley, *Hordeum jubatum* L., dwarf plantain, *Plantago Purshii* R. & S., blue bur, *Lappula echinata* Gilib., and gum weed, *Grindilia perennis* A. Nels. The area surrounding this site is typical short-grass prairie dominated by *Bouteloua gracilis* and *Stipa comata*. In November of 1928, the area was seeded to equal sized plots of the following grasses: slender wheatgrass, *Agropyron trachycaulum* var. *typicum*, crested wheatgrass, blue joint, *Agropyron Smithii*, and brome grass, *Bromus inermis*. Of these species the only one surviving in 1948 was the crested wheatgrass. It had been broadcast at 16 pounds to the acre and then single disked. Half of the area seeded to crested wheatgrass was fenced off to prevent grazing. The other half has been heavily spring grazed since it was planted. In 1948, the area had only been lightly grazed and the exclosure, of course, had not been touched. Both areas were point sampled and plots were clipped during the summer of 1948. The results are presented in Table 3.

TABLE 3.—RESULTS OF POINT SAMPLING AND CLIPPINGS ON SITE 1—DENSITY OF VEGETATIVE COVER EXPRESSED AS A PERCENTAGE OF GROUND AREA

	Crested wheatgrass	Stipa comata	Bouteloua gracilis	Koeleria cristata	Poa secunda	Others	Total	Total yield (lb./acre)
Inside exclosure	4.7	0.1	0.2	0.5	0.2	—	5.7	457
Outside exclosure	3.1	1.2	3.0	0.1	3.0	—	7.8	376
Native pasture	—	2.1	3.7	0.8	0.2	1.6*	8.4	300

* 1.0 Per cent is *Agropyron Smithii*, the remaining 0.6 others.

The information presented in Table 3 indicates that, when crested wheatgrass is grazed early in the spring, the native dominants will advance and compete successfully, if a plentiful supply of seed is available. Thus, old stands that are heavily grazed may be invaded by pasture weeds, unless good management practices are followed. However, the crested wheatgrass provided an excellent cover and high quality spring pasture for 20 years, and to-day the grazed area outside the exclosure far outyields the native range on adjacent lands.

Site 2 is located two and one-half miles south of the Station. It was drilled in 6-inch rows during April, 1932, to crested wheatgrass at the rate of 16 pounds per acre. This field has been ploughed and harrowed in August of 1931 after growing a crop of weeds, chiefly Russian thistle and tumbling mustard. The field was protected for one year following seeding, and has been grazed lightly and cut for hay since that date.

Site 2 was point sampled this year, and plots were clipped to estimate yields. The density of crested wheatgrass expressed in per cent was 5.1 per cent; and the yield 359 pounds per acre. At present, there is very little sign of invasion by native grasses. Also there are very few plants of crested wheatgrass that have spread into the native prairie surrounding the reseeded area.

Site 3 is located approximately five miles west of the Station. Part of the field had produced a crop of rye in 1935, whereas the remainder had been abandoned in 1933. Crested wheatgrass was seeded in 1936 using different cultural treatments and different rates of seeding.

On the east side of the field plots were seeded by the broadcast method at the rate of 3 and 6 pounds per acre, on November 2, 1936. One-half of each plot was single disked. Light, scattered stands were produced on all plots. In 1938, meter quadrats were established to study the rate of increase in density of cover. During August of 1940 and 1948, these quadrats were recharted. The information is presented in Table 4.

TABLE 4.—COVERS DETERMINED BY AREA-LIST AND POINT SAMPLING METHOD, AND YIELD FROM CLIPPED QUADRATS ON BROADCAST PLOTS AT SITE 3

	Year sampled	Rate of seeding lb. per acre	Average number plants per sq. meter	Per cent density quadrat method	Per cent density point method	Yield lb./acre air dry
1. Treatment— Broadcast and single disked	1938	1	38	1.50	—	—
		3	23	1.50	—	—
		6	—	—	—	—
	1940	1	48	4.62	—	—
		3	93	5.12	—	—
		6	—	—	—	—
	1948	1	44	4.28	4.9	387
		3	43	4.66	5.1	346
		6	41	4.36	5.0	303
2. Treatment— Broadcast	1938	1	10	0.80	—	—
		3	39	2.02	—	—
		6	21	1.75	—	—
	1940	1	22	4.05	—	—
		3	208	6.72	—	—
		6	148	7.0	—	—
	1948	1	52	6.15	5.2	361
		3	33	4.31	4.6	322
		6	45	4.35	5.1	335

Three widths of row spacing were seeded in April, 1936, west of the broadcast plots. The spacings tested were 6, 12, and 24 inches between rows (Figures 2, 3, and 4).

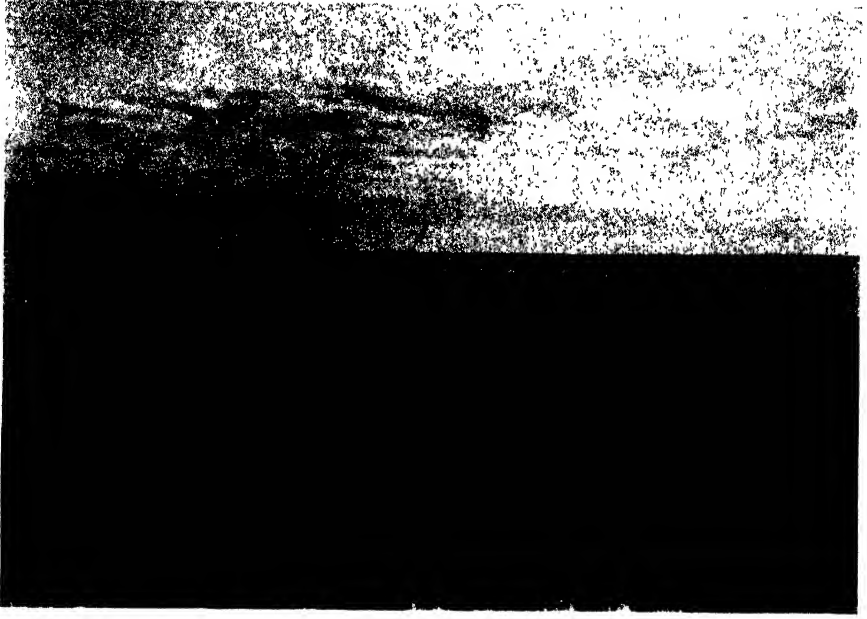


FIGURE 2. Close drilled crested wheatgrass seeded in 1936—Site 3.



FIGURE 3. Twelve-inch spacing of crested wheatgrass sown in 1936—Site 3.

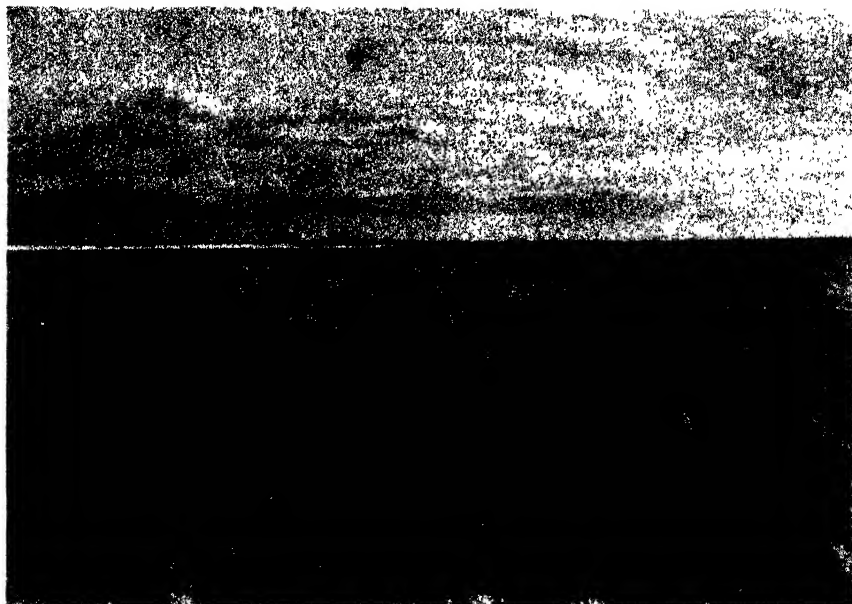


FIGURE 4 Twenty-four inch spacings of crested wheatgrass sown in 1936. Note the filling in between the rows—Site 3.



FIGURE 5. Crested wheatgrass sown into native sod in background. Notice on the extreme left the dense stand of crested wheatgrass, while native vegetation dominates the plot on the right.

Comparisons of the yields and densities of the different rates of seeding, different cultural treatments, and different row spacings are presented in Table 5.

TABLE 5.—COMPARISON OF YIELDS AND DENSITIES ON SITE 3, 1948

Treatment	Date seeded	1948 density	Yield—lb./acre	
			1940	1948
Drilled 6-inch spacing, 12 lb./acre	April, 1936	5.4	1082	353
Drilled 12-inch spacing, 6 lb./acre	April, 1936	5.3	1054	363
Drilled 24-inch spacing, 3 lb./acre	April, 1936	5.6	736	403
Broadcast—single disk, 6 lb./acre	November, 1936	4.4	—	303
Broadcast—single disk, 3 lb./acre	November, 1936	4.7	—	346
Broadcast—single disk, 1 lb./acre	November, 1936	4.3	—	387
Broadcast—6 lb./acre*	November, 1936	4.3	—	335
Broadcast—3 lb./acre*	November, 1936	4.3	—	322
Broadcast—1 lb./acre*	November, 1936	6.2	—	361
Average		4.9	—	352

* No cultural treatment.

The information in Tables 4 and 5 shows that after a 10-year period a state of equilibrium is reached no matter what cultural practice, seeding method, or rate of seeding is employed, providing there is a favourable seed-bed in which to sow the grass. Under the prevailing climatic conditions, only a certain number of plants will grow, and for each specific year a similar yield can be expected, regardless of rates of seeding or cultural treatments after a stand has been obtained.

In 1940, the plots at Site 3 yielded an average of 1,117 pounds per acre, which high yield is credited to the excellent growing conditions of the season, as well as the youth and vigour of the stand. In 1948, the yields presented in Table 5 are much lower but are not significantly different for either treatment or seeding. During one year of better than average moisture conditions, it is doubtful if the density of the cover would change. However, if improved growth conditions continued for a period of two or more years an increase in stand might be expected. It is interesting to note that the different cultural treatments and the different rates of seeding appear to have no effect on the density or yield of stands produced 12 years after seeding. It may be that this effect is apparent before this length of time has elapsed, but unfortunately no sampling was conducted on any of the areas, and only a few yields were taken prior to 1948. At Mandan, North Dakota, workers have found that thick stands tended to remain thick but the individual plants produced were inclined to be stunted (4).

REGRASSING OF SPRING FLOODED MEADOWS

At Site 4, the benefits of spring flooding were studied. This site is located about eight miles northwest of the Station. It is a heavy clay flat that has been dyked to use the spring run-off. In 1938, a seed^bbed was prepared and seeded to crested wheatgrass in both 6-inch and 12-inch rows. Table 6 presents the results of the point sampling conducted in 1948.

TABLE 6.—DENSITY AND YIELD OF CRESTED WHEATGRASS STANDS
AT SITE 4—AUGUST, 1948

Spacing	Rate, lb. per acre	Date seeded	Density	Yield, lb. per acre
6-inch	12	November, 1938	6.7	1,627
12-inch	6	November, 1938	7.0	1,696
Average	—		6.8	1,661

In comparing the densities of the stands with those of Sites 3 and 4, it will be observed that the density of crested wheatgrass at Site 4 is only increased 39 per cent more than that at Site 3, yet the average yield is increased over 370 per cent. It is interesting to note that at Site 4 the area seeded in 12-inch rows, at the rate of six pounds per acre, had a denser cover (though insignificantly higher), and yielded more than the closer spaced seeding sown at double the rate. Except for the annual rainfall, the only other water this area receives is from the spring run-off which is spread by a dyking system. Thus spring flooding appears to increase the yield many times without greatly increasing the ground cover. Such is a useful grass character for spring flooded projects where, during one out of each three years, there will be either no run-off or insufficient to obtain a satisfactory flooding. When such occurs, crested wheatgrass stands were seldom depleted because of drought, and thus resist the rapid encroachment of pasture and hayland weeds.

RESEEDING ON NATIVE PRAIRIE

An experiment was designed to determine the best method of seeding depleted rangeland to crested wheatgrass. In April, 1938, six treatments were established. These were:

- Treatment 1—Single disked and crested wheatgrass broadcast
- Treatment 2—Crested wheatgrass broadcast then single disked
- Treatment 3—Single disked, crested wheatgrass drilled 6-inch spacing
- Treatment 4—Single disked, crested wheatgrass drilled 12-inch spacing
- Treatment 5—Crested wheatgrass drilled 6-inch spacing
- Treatment 6—Crested wheatgrass drilled 12-inch spacing.

The covers of the treatments were studied by the use of a meter quadrat frame in 1947. The results are listed in Table 7.

Prior to the time of seeding, the site had been overgrazed and was in a badly depleted condition. Annual weeds were beginning to invade the area. Following seeding, grazing was continued but at a much reduced rate. Because of adverse climatic and ecological conditions, only a very sparse cover of crested wheatgrass is present after nine years. However, the information indicates that the more the ground was worked prior to seeding, which means the killing of native plants, the better the crested wheatgrass cover. Although a stand is being established, it is a very slow process. After eight years, the cover is less than one per cent with the best treatment employed. With a good seed-bed, annual weeds, or stubble, the cover would be from six to eight times greater within a three-year period.

TABLE 7.—PER CENT COVER OF CRESTED WHEATGRASS SEEDED INTO OVERGRAZED NATIVE PASTURE NINE YEARS AFTER SEEDING, 1939-47

Dominion Range Experiment Station, Manyberries, Alberta

	Treatment					
	1	2	3	4	5	6
Number of crested wheatgrass plants	167	72	173	64	25	3
Total surface area sq. cm.	125,000	125,000	125,000	125,000	125,000	125,000
Area covered by crested wheatgrass plants in sq. cm.	1,209	332	771	625	59	37
Crested wheatgrass cover as a percentage of ground area	0.96	0.26	0.61	0.50	0.04	0.03

An interesting observation was made this year at the North Montana Branch Experiment Station, Havre, Montana. During the worst years of the drought, it was decided to try sowing crested wheatgrass directly into the native sod without any previous preparation (Figure 5).

The experiment was started in 1936 and continued till 1940. In 1936, the vigour of the native vegetation was at an exceedingly low ebb, making conditions optimum for the establishment of a stand of crested wheatgrass. As climatic conditions became more favourable for the native vegetation, the stands of crested wheatgrass obtained diminished. The first year of the seeding trial an almost 100 per cent stand was obtained. In 1940, only a few plants of crested wheatgrass were evident. This area has had complete protection from grazing since the experiment was started. The results would seem to indicate that crested wheatgrass may be established on native prairie by simply seeding with a drill, if ecological conditions are favourable to its establishment. These conditions may be brought about by mechanical means such as by using the Noble blade or disking.

In 1938, a field of overgrazed native pasture at the Dominion Experimental Station, Swift Current, Saskatchewan, was drilled in 12-inch rows to crested wheatgrass, at the rate of 8 pounds to the acre. Quadrats were established on this area in 1946. These were charted by the area-list method and have been recharted in 1947 and 1948 by the same method. The results are presented in Table 8.

TABLE 8.—SUMMARY OF QUADRAT INFORMATION FROM 1938 SEEDING, DOMINION EXPERIMENTAL STATION, SWIFT CURRENT, SASK.

Year of charting	1946	1947	1948	
Average number of plants of crested wheatgrass per m ²	20.3	17.7	24.4	Ungrazed
Total basal area of crested wheatgrass plants per m ²	167.0	227.1	256.5	
Crested wheatgrass cover as a percentage of ground area	1.67	2.27	2.565	
Average number of plants of crested wheatgrass per m ²	1.1	2.3	4.0	Grazed
Total basal area of crested wheatgrass plants per m ²	1.9	5.8	8.0	
Crested wheatgrass cover as a percentage of ground area	0.019	0.058	0.08	

Previous to 1938, there were no plants of crested wheatgrass on the native sod. The results presented in Table 8 show that crested wheatgrass had developed a two and one-half per cent cover during a period of 10 years under complete protection. When grazed, the cover of crested wheatgrass is increasing, but much more slowly.

SUMMARY AND CONCLUSIONS

1. Seed bed, stubble or annual weeds. Native sod not suitable unless the cover is depleted by drought or cultivation.

2. Heavy spring grazing on old stands of crested wheatgrass has resulted in an invasion of other grasses when a source of seed was readily available. The invasion did not occur to the same extent if the crested wheatgrass was protected. There was no invasion of the crested wheatgrass into adjacent native sod.

3. If the seed-bed is suitable, there will be no significant difference after a 10-year period in density of stand or yields when seedings are either broadcast or seeded, and when seeded at rates of 1 to 12 pounds per acre.

4. Spring flooding does not greatly increase the ground cover over that on dryland, but increases the yield by three to four times.

5. Reseedings on native sod will not be successful unless the native cover is destroyed by either drought or cultivation. Protection increases the rate of advance of crested wheatgrass into native sod.

Crested wheatgrass is a hardy perennial grass, well suited to the climatic conditions of Southern Alberta and Saskatchewan, as shown by the experiments conducted by the Dominion Range Experiment Station, Manyberries, Alberta. Heavy grazing is detrimental to stands of crested wheatgrass the first year after seeding before the plants are able to establish themselves. During this period, overgrazing weakens the crested wheatgrass plants to the extent that natural succession takes place more rapidly than in exclosures. When crested wheatgrass has been seeded on overgrazed native prairie, the per cent density of stand appears to be controlled by the condition and vigour of the native vegetation. However, in no case does the stand of crested wheatgrass seem to be decreasing, and in most cases it is increasing, even though very slowly, especially where grazing is practised.

The information indicates that over a period of ten years either the rate of seeding or the cultural method used will have little or no effect on either the density or yield of a stand. In a wet year, the density of the stand will not change to any extent, but the yield may increase considerably. This applies to spring flooded areas where the density of the grass is only increased by 39 per cent, while the yield is increased 370 per cent over crested wheatgrass grown on dryland.

Yields of crested wheatgrass do not appear to follow any definite curve from year to year. Yield seems to depend on available moisture which (1) may triple the amount of hay, or (2) under dry conditions tends to kill out the weaker plants.

ACKNOWLEDGMENTS

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THE SURVIVAL OF GRASSHOPPER NYMPHS ON VEGETATION TREATED WITH 2,4-D¹

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Fox (1) has drawn attention to a relationship between the use of the selective herbicide 2,4-D and damage to wheat caused by the wireworm *Ctenicera aeripennis destructor* Brown. Where 2,4-D was applied for weed control just prior to the seeding of wheat, the amount of damage the wireworms inflicted on the crop was increased. As the use of 2,4-D for weed control may affect other insects, it seems desirable to record some observations on the effect of this chemical on the survival of grasshoppers, mostly nymphal *Melanoplus m. mexicanus* Sauss. Any environmental factor that alters the percentage survival of this species is of great importance, since the mortality in the early stages is normally high. For example, a small percentage increase or decrease might, on the one hand, double the numbers reaching maturity, or, on the other hand, reduce them to one-half.

The observations were made in 1947 on a field experiment of which the 2,4-D treated plots formed one part. The experiment was laid out on 4 strips of wheat stubble, each one mile long, and all situated on one farm near Milo, Alberta. There were 16 four-acre plots, of which 8 were treated with 2,4-D, and 8 were untreated checks. These were distributed over the experimental area at random, but with local control so that 2 treated and 2 untreated plots were placed on each strip. The experimental area was also divided transversely into two equal blocks, and the distribution of the plots was so controlled that half the replicates were in each block.

The herbicide used was an emulsion concentrate of the butyl ester of 2,4-D, said to contain 32 per cent of the acid equivalent. It was applied with a turbine sprayer at the rate of 16 fluid ounces per acre in about 4 gallons of water. Treatment was carried out on June 16, 1947.

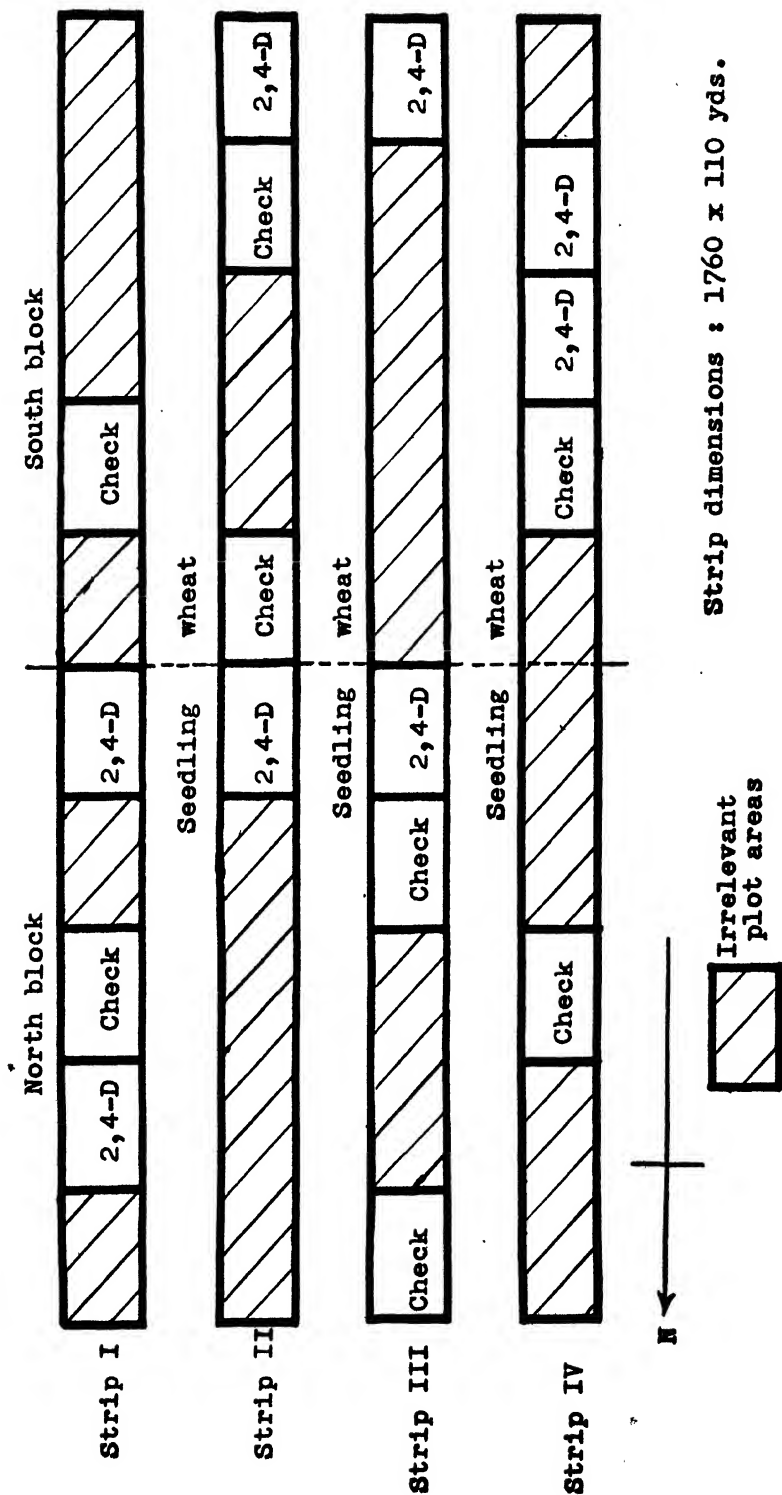
At the time of treatment, the main green vegetation on the plots consisted of stinkweed (*Thlaspi arvense* L.) in flowering and early fruiting stage, Russian thistle (*Salsola pestifer* (A. Nels.) in the succulent foliaceous stage, lambs' quarters (*Chenopodium album* L.) in vegetative to early blooming stage, and tumbling mustard (*Sisymbrium altissimum* L.) in rosette stage. There was little volunteer wheat or other graminaceous species present, which are superficially unaffected by dosages of 2,4-D sufficient for killing the species of weeds listed. Therefore, any grasshoppers present had to feed on weeds, which soon showed the characteristic symptoms following treatment. With the exception of a few tumbling mustard rosettes, the affected plants remained green for the duration of the experiment, up to July 2.

The evaluation of the grasshopper populations was made by 16 separate visual estimates of numbers per square yard on each plot. The sampling procedure was designed to ensure a population estimate reasonably repre-

¹ Contribution No. 2606 from the Division of Entomology, Science Service, Department of Agriculture. This experiment was part of the Prairie Regional Grasshopper Project, under the leadership of R. H. Handford.

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PLAN OF EXPERIMENTAL PLOTS : SURVIVAL OF GRASSHOPPER
NYMPHS ON VEGETATION TREATED WITH 2,4-D



sentative of the whole plot. Only one complete appraisal of the whole experiment was made, on June 24. Part of the experiment was available for a second appraisal on July 2.

Hatching of *M. mexicanus* eggs occurred later than usual in 1947. At the time the treated plots were sprayed, grasshopper nymphs were very scarce, and when the experiment had to be abandoned on July 2, nymphal development and egg data suggested that hatching was still incomplete. Observations had to be carried out on a basis of very small numbers so that the proportional variability in results was very high.

On June 24, when the first estimates of abundance were made, the average population on the treated plots was estimated at 0.46 per sq. yd., while that on the untreated plots was only 0.23 per sq. yd. When the final observation was made on July 2 on the one remaining strip of the experimental area, the treated plots averaged 4.2, and the untreated plots 2.3 nymphs per sq. yd. The size of the differences, and the degree to which they were consistently observed, would suggest significance, but it was not possible to show this in a formal variance analysis, because the variability from plot to plot was too great.

Tables 1 and 2 are presented in order to indicate the degree to which the ratio between populations on treated and untreated areas varied or was consistent. Table 1 shows how the ratio varied from strip to strip, and that, although there was wide variation, the average population on the treated portions always exceeded that on untreated portions of any given strip. Table 2 shows that when the relative populations on the two *blocks* were considered, they were almost identical both in proportion and in absolute numbers.

TABLE 1.—ESTIMATED GRASSHOPPER POPULATIONS ON 2,4-D TREATED AND UNTREATED VEGETATION WITH REFERENCE TO STRIPS

(Populations per sq. yd. \times 32)

Strip	I		II	III	IV	Total
Date	June 24	July 2	June 24	June 24	June 24	June 24
Treated, 2-4-D	23	134	10	15	11	59
Check	13	73	5	2	10	30

TABLE 2.—ESTIMATED GRASSHOPPER POPULATIONS ON 2,4-D TREATED AND UNTREATED VEGETATION, WITH REFERENCE TO BLOCKS

(Population per sq. yd. \times 64, as of June 24)

	North block	South block	Total
Treated, 2,4-D	30	29	59
Check	15	15	30

While no formal claims can be made at this time regarding the apparent observation that treatment of weeds with 2,4-D hastens the development or increases the percentage survival of *M. mexicanus*, the evidence seems sufficient to show that such treatment does not depress survival. In fact, it was observed that the most densely populated area in the whole experiment was a patch of lambs' quarters showing typical symptoms of the action of 2,4-D. Therefore, from the point of view of grasshopper control, the use of 2,4-D alone to control weeds, just prior to or during grasshopper hatching, could be a dangerous procedure as compared with some recommended tillage operations carried out in the fall or early spring. This would apply whether 2,4-D treatment is advantageous to grasshoppers or whether it merely does not interfere with them as long as the affected plants remain succulent. It is therefore suggested at this time that where "chemical summer-fallowing" is deemed necessary in the control of soil erosion, and where at the same time grasshoppers are a threat, persons who intend to try chemical summer-fallowing should be prepared to control grasshoppers by means of insecticides.

This work does not establish whether or not any increase in numbers of grasshoppers found in treated vegetation could be a nutritional effect, resulting from changes in the physiology of the food plants. An alternative possibility has been considered—that the distorted plants fail to shade the ground as fully as normal plants. This could result in a higher soil temperature and an accelerated rate of hatching.

Green weeds are probably important to ovipositing *M. mexicanus* in stubble fields. If the general use of herbicides were to result ultimately in the nearly complete removal of susceptible weeds from cereal crop ecology, the long-term effects on grasshoppers might differ from the immediate ones forecast here.

ACKNOWLEDGMENTS

A. H. Carter, of "Green Cross" Insecticides Division, The Sherwin Williams Co., kindly made sufficient 2,4-D available for the experiment. Roy O'Leary, of Queenstown, Alberta, co-operated in an essential way by providing the land necessary for the experimental lay-out.

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DIGESTIBILITY STUDIES WITH RUMINANTS

XIV. THE EFFECT OF THE PLANE OF NUTRITION ON THE DIGESTIBILITY OF BARLEY¹

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In a previous publication (4) the effect of the level of feeding on the digestibility of rations of equal parts of hay and barley was determined. Using grade Shorthorn steers the total ration was fed at levels of 2, 4, 6.5, 9 and 10 kg. per animal per day. It was found that the digestibility of nitrogen decreased as the plane of nutrition was increased. The total decrease amounted to about 6 absolute per cent and the difference was statistically significant. The digestibility of the nitrogen free extract showed a small decrease of 1.5 absolute per cent which was statistically significant. The digestibilities of the other nutrients did not show much change with the increase in plane of nutrition.

When the coefficients of the barley were calculated from the mixed rations with hay the decreases were intensified. For instance, in the case of the total digestible nutrients the respective values for the 5 increasing levels were 83.6, 84.0, 84.5, 81.9 and 80.4 per cent of the dry matter. This decrease was statistically significant. Any effect of the plane of nutrition on the digestibility of the mixed rations due to the presence of barley would be masked to a certain extent by the presence of an equal amount of hay. It was shown in a previous publication (3) that the plane of nutrition has no effect on the digestibility of hay. To determine the effect on the digestibility of barley the present experiment was carried out. Hay was fed at a constant level and barley at increasing levels. At the same time the coefficients of digestibility of the hay alone were determined.

EXPERIMENTAL

Digestion trials were carried out with six grade Shorthorn steers. They were 1½-2 years old and weighed on an average, throughout the experiment, 430 kilograms. They were numbered 1-6, respectively. Six rations were used. Hay was fed alone at approximately 7 kg. (the average of all six animals was 6.8 kg.). In the mixed rations hay was fed at a level of 3 kg. Various rations were made up by feeding barley at 1, 2, 3, 4 and approximately 5 kg. per day. The quantity of barley given at the highest level of feeding for each animal was based on the maximum amount which that animal could consume. The average of the six trials at the maximum level was 4.73 kg. per animal per day.

The barley was Western Canada Feed No. 1. The hay was largely timothy. The botanical composition is shown in Table 1. The chemical composition of the feeds is given in Table 2.

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TABLE 1.—BOTANICAL ANALYSIS OF MIXED HAY

Type of herbage	Per cent
Timothy	65.7
Legumes (red and alsike clover, alfalfa)	33.4
Couch grass	0.7
Miscellaneous	Trace

TABLE 2.—CHEMICAL COMPOSITION OF FEEDS (MEANS OF 6 VALUES)

Feed	Original dry matter %	Composition of dry matter				
		Ash %	Protein* %	Ether extract %	Crude fibre %	N-free extract %
Hay	87.14	5.49	9.85	1.00	38.04	45.62
Barley	86.01	2.92	12.80	1.87	6.62	75.78

* Protein factors (Jones (2))

Hay - $N \times 6.25$

Barley - $N \times 5.83$.

The experiment was set up in the form of a 6×6 randomized Latin square, a different ration being fed to each animal in each of six periods. The collection period was 12 days in length preceded by a preliminary period of 16 days.

Progress of Experiment

There were some feed refusals. In period 2, animal 4 receiving 4 kg. of barley refused some feed and a total of two rations was removed during the first 8 days of the collection period. The results for this animal were calculated on the basis of a 10-day collection period. In the same period animal 6 receiving the maximum quantity of barley refused some feed at the beginning of the preliminary period. It was necessary to adjust the ration for this animal, lengthen the preliminary period and reduce the collection period to 10 days. In period 4, animal 1 receiving the maximum quantity of barley refused its ration on the fourth day of the collection period. Otherwise the ration was consumed satisfactorily. The results in this case were calculated on the basis of an 11-day collection period. Apart from these instances the experiment proceeded satisfactorily. The rations were consumed regularly and all the animals maintained good health.

RESULTS

In Appendix Table 6 the coefficients of digestibility of the total rations for dry matter and nitrogen have been arranged in a Latin square according to ration, period and animal. An analysis of variance is given in Table 7. The data are summarized in Table 3. This table gives the mean coefficients of digestibility of the dry matter and of the nitrogen for each animal and for each period. The "F" values have been included.

TABLE 3.—MEAN COEFFICIENTS OF DIGESTIBILITY OF THE DRY MATTER AND NITROGEN OF THE TOTAL RATIONS ARRANGED BY PERIODS AND BY ANIMALS (MEANS OF SIX VALUES)

Coefficients of digestibility of dry matter				Coefficients of digestibility of nitrogen			
Arranged by animals		Arranged by periods		Arranged by animals		Arranged by periods	
Animal No.	Coefficient %	Period No.	Coefficient %	Animal No.	Coefficient %	Period No.	Coefficient %
1	65.8	1	66.3	1	64.0	1	65.1
2	64.4	2	65.2	2	60.6	2	65.0
3	64.6	3	63.9	3	63.4	3	62.9
4	65.9	4	64.5	4	64.4	4	61.8
5	63.7	5	65.1	5	62.2	5	64.3
6	65.0	6	64.3	6	64.8	6	60.3
F value*	1.09	F value*	1.15	F value*	2.50	F value*	3.68

* Nec. F calculated when 6 rations, 6 animals and 6 periods are arranged in a Latin square, is 2.71 at $P = 0.05$ and 4.10 at $P = 0.01$.

It is apparent that there was no significant difference between the animals in their ability to digest the dry matter and nitrogen. There was no significant difference between the periods for dry matter. There was a significant difference between the periods for nitrogen. Periods 1, 2 and 5 were slightly higher than periods 3, 4 and 6. In each case the three periods were similar. There was no particular trend throughout the course of the experiment.

The coefficients of digestibility of the hay and of the barley calculated from the various hay rations are given in Appendix Table 8, with an analysis of variance in Appendix Table 9. In Appendix Table 10 are given the percentage digestibilities of the gross energy of the hay and of the barley calculated from the rations with hay. The analysis of variance is given in Appendix Table 11. All these results have been summarized in Table 4, which includes the necessary differences for significance.

TABLE 4.—MEAN COEFFICIENTS OF DIGESTIBILITY OF NUTRIENTS OF BARLEY CALCULATED FROM MIXED RATIONS WITH HAY. (MEANS OF SIX VALUES)

Ratios of hay to barley in rations		Mean coefficients of digestibility of following nutrients						T.D.N. in per cent of dry matter
Hay	Barley	Dry matter	Organic matter	Nitrogen	Ether extract	Total carbohydrates	Gross energy	
Kg.	Kg.	%	%	%	%	%	%	
3	1	85.0	87.6	73.2	80.0	89.9	85.0	87.5
3	2	81.8	83.7	74.6	84.6	85.2	81.9	84.0
3	3	80.0	80.0	72.8	80.5	83.5	79.4	82.0
3	4	77.0	78.9	70.7	74.2	80.3	76.0	79.0
3	5*	73.3	75.1	66.7	61.1	76.8	72.2	75.0
Nec. diff. at $P = 0.05$		5.8	5.6	5.9	11.5	5.7	6.0	5.5

* Approximately according to capacity of animals.

It is evident from this table that the digestibility was lowest at the maximum level of feeding. With the exception of nitrogen and ether extract it was highest at the lowest level of feeding. In the case of these two nutrients there was no statistically significant change in the three lower levels. Then the digestibility decreased. These results confirm the findings of the previous paper (4). The differences are of a larger magnitude in view of the fact that barley alone was increased.

The levels of feeding were translated into terms of planes of nutrition by using Brody's tables of maintenance requirements (1). For each ration the weights of the six animals were averaged and their requirements in total digestible nutrients and digestible protein estimated according to Brody's formulae. The daily consumption for each ration was averaged for the six animals and the content in T.D.N. and digestible protein determined. These values have been expressed in Table 5.

TABLE 5.—PLANE OF NUTRITION EXPRESSED IN TERMS OF MAINTENANCE REQUIREMENTS USING BRODY'S FORMULAE (1). (MEANS FOR SIX ANIMALS ON EACH RATION)

Ration		Daily nutrients required and consumed					
Hay	Barley	T.D.N.			Digestible protein		
		Consumed daily	Required	Per cent consumed of requirements	Consumed daily	Required	Per cent consumed of requirements
Kg.	Kg.	Kg.	Kg.		Gm.	Gm.	
6.5	0	3.20	3.04	105	318	316	101
3	1	2.17	2.88	75	226	299	76
3	2	2.86	2.91	98	317	302	105
3	3	3.54	2.94	120	398	305	130
3	4	4.13	2.98	139	474	309	153
3	5*	4.46	3.01	148	512	312	164

* Approximately, according to capacity of animals.

It will be observed that for the T.D.N., the hay ration was fed at approximately the maintenance level and the barley-hay rations at from 0.75 to approximately 1.5 maintenance. As far as digestible protein was concerned the hay ration was fed at approximately the maintenance level and the barley-hay rations at levels of from approximately 0.75 to 1.6 maintenance.

SUMMARY AND CONCLUSIONS

1. Six grade Shorthorn steers were fed rations of hay alone and of hay with increasing quantities of barley.
2. The hay was fed at approximately maintenance level and the mixed rations were fed at levels of approximately 0.75 to 1.5 maintenance.
3. The coefficients of digestibility of barley were calculated from the mixed rations with hay.
4. It was found that as the plane of nutrition increased the digestibility of the barley decreased.

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APPENDIX

TABLE 6.—COEFFICIENTS OF DIGESTIBILITY OF DRY MATTER AND NITROGEN OF TOTAL RATIONS ARRANGED BY RATIONS, PERIODS AND ANIMALS (ANIMAL NUMBERS SHOWN IN BRACKETS, COEFFICIENTS IN PER CENT)

Nutrient	Period No.	Coefficient of following rations					
		Hay	Mixtures of hay : barley				
			3 : 1	3 : 2	3 : 3	3 : 4	3 : max.
Dry matter	1	57.0 (2)	64.5 (1)	67.4 (3)	67.5 (5)	68.7 (4)	72.8 (6)
	2	56.6 (3)	67.0 (4)	65.6 (5)	70.0 (1)	69.4 (6)	62.7 (2)
	3	55.6 (6)	62.1 (3)	67.5 (1)	67.7 (2)	63.9 (5)	66.5 (4)
	4	55.9 (5)	61.1 (6)	66.9 (2)	68.1 (4)	67.9 (3)	67.1 (1)
	5	56.6 (1)	63.7 (5)	67.9 (4)	67.5 (6)	69.6 (2)	65.1 (3)
	6	57.0 (4)	62.5 (2)	63.7 (6)	68.2 (3)	68.8 (1)	65.5 (5)
Nitrogen	1	56.9 (2)	61.1 (1)	66.5 (3)	67.8 (5)	66.7 (4)	71.4 (6)
	2	62.2 (3)	67.4 (4)	64.7 (5)	69.3 (1)	67.5 (6)	59.1 (2)
	3	55.8 (6)	61.1 (3)	67.6 (1)	64.0 (2)	63.6 (5)	65.1 (4)
	4	53.2 (5)	63.2 (6)	61.0 (2)	65.1 (4)	66.2 (3)	61.9 (1)
	5	58.8 (1)	61.7 (5)	69.6 (4)	68.0 (6)	65.7 (2)	62.0 (3)
	6	52.7 (4)	56.6 (2)	62.9 (6)	62.2 (3)	65.2 (1)	62.0 (5)

TABLE 7.—ANALYSIS OF VARIANCE OF DATA IN TABLE 6†

Nutrient	Variance due to	D/F	Sums of squares†	Variance†	F
Dry matter	Ration	5	597.04	119.408	*
	Period	5	22.24	4.45	1.15
	Animal	5	21.12	4.22	1.09
	Error	20	77.20	3.86	—
Nitrogen	Ration	5	392.16	78.43	12.78
	Period	5	112.94	22.59	3.68
	Animal	5	76.87	15.37	2.50
	Error	20	122.78	6.14	—

† Nec. F. for $N_1 = 5$, $N_2 = 20$ at P of 0.05 = 2.71.

Nec. F. for $N_1 = 5$, $N_2 = 20$ at P of 0.01 = 4.10.

* Obviously significant.

† Calculated to four decimal places, reported to two.

TABLE 8.—COEFFICIENTS OF DIGESTIBILITY OF NUTRIENTS IN HAY AND OF NUTRIENTS IN BARLEY CALCULATED FROM MIXED RATIONS WITH HAY, ARRANGED BY RATIONS, PERIODS AND ANIMALS. (ANIMAL NUMBERS IN BRACKETS, COEFFICIENTS IN PER CENT)

Nutrient	Period No.	Coefficients of digestibility					
		Hay	Barley calculated from the following ration with hay				
			H-3 B-1	H-3 B-2	H-3 B-3	H-3 B-4	H-3 B-Max.
Dry matter	1	57.0 (2)	89.4 (1)	84.4 (3)	78.9 (5)	78.2 (4)	84.8 (6)
	2	56.6 (3)	99.1 (4)	79.6 (5)	83.7 (1)	79.2 (6)	66.6 (2)
	3	55.6 (6)	79.3 (3)	84.3 (1)	79.2 (2)	69.7 (5)	72.9 (4)
	4	55.9 (5)	75.3 (6)	82.9 (2)	79.8 (4)	76.7 (3)	73.6 (1)
	5	56.6 (1)	85.8 (5)	85.3 (4)	78.6 (6)	79.7 (2)	70.6 (3)
	6	57.0 (4)	80.8 (2)	74.6 (6)	80.0 (3)	78.2 (1)	71.2 (5)
Organic matter	1	58.4 (2)	93.4 (1)	86.3 (3)	80.2 (5)	80.9 (4)	85.9 (6)
	2	57.5 (3)	102.2 (4)	81.5 (5)	86.0 (1)	81.0 (6)	69.4 (2)
	3	56.5 (6)	82.3 (3)	86.2 (1)	81.3 (2)	71.2 (5)	74.8 (4)
	4	57.0 (5)	77.4 (6)	84.8 (2)	82.0 (4)	78.9 (3)	75.5 (1)
	5	57.6 (1)	86.7 (5)	87.1 (4)	80.6 (6)	81.6 (2)	72.3 (3)
	6	58.3 (4)	83.3 (2)	76.3 (6)	82.0 (3)	79.9 (1)	72.9 (5)
Nitrogen	1	56.9 (2)	71.0 (1)	77.2 (3)	76.0 (5)	72.1 (4)	79.1 (6)
	2	62.2 (3)	92.4 (4)	74.0 (5)	79.1 (1)	73.8 (6)	60.3 (2)
	3	55.8 (6)	69.5 (3)	77.8 (1)	68.6 (2)	66.9 (5)	68.4 (4)
	4	53.2 (5)	76.5 (6)	65.4 (2)	70.9 (4)	71.1 (3)	64.0 (1)
	5	58.8 (1)	73.5 (5)	84.5 (4)	76.7 (6)	70.9 (2)	64.5 (3)
	6	52.7 (4)	56.3 (2)	69.0 (6)	65.8 (3)	69.4 (1)	64.1 (5)
Ether extract	1	35.2 (2)	55.8 (1)	88.1 (3)	82.0 (5)	58.7 (4)	70.3 (6)
	2	30.3 (3)	94.1 (4)	84.4 (5)	79.2 (1)	80.9 (6)	40.8 (2)
	3	34.1 (6)	97.5 (3)	90.6 (1)	86.8 (2)	81.2 (5)	71.8 (4)
	4	25.0 (5)	82.0 (6)	86.6 (2)	83.1 (4)	77.4 (3)	81.7 (1)
	5	17.2 (1)	83.0 (5)	86.3 (4)	70.3 (6)	77.6 (2)	61.6 (3)
	6	9.6 (4)	67.6 (2)	71.3 (6)	81.7 (3)	69.3 (1)	60.2 (5)
Crude fibre	1	55.9 (2)	129.2 (1)	46.2 (3)	-8.5 (5)	21.2 (4)	29.7 (6)
	2	50.3 (3)	136.0 (4)	-8.7 (5)	36.0 (1)	-11.7 (6)	-43.8 (2)
	3	53.1 (6)	50.7 (3)	50.7 (1)	20.5 (2)	-47.5 (5)	-7.8 (4)
	4	51.0 (5)	-95.2 (5)	55.3 (2)	5.2 (4)	-8.4 (3)	-6.2 (1)
	5	52.1 (1)	3.4 (5)	5.4 (4)	3.3 (6)	24.1 (2)	-50.5 (3)
	6	58.1 (4)	99.3 (2)	-16.0 (6)	31.9 (3)	39.7 (1)	-8.5 (5)
Nitrogen free extract	1	61.0 (2)	94.3 (1)	91.0 (3)	87.7 (5)	87.3 (4)	91.6 (6)
	2	62.2 (3)	101.1 (4)	90.3 (5)	91.3 (1)	90.1 (6)	81.3 (2)
	3	59.6 (6)	88.7 (3)	91.2 (1)	88.8 (2)	82.3 (5)	83.5 (4)
	4	62.1 (5)	91.8 (6)	90.2 (2)	90.6 (4)	87.4 (3)	84.7 (1)
	5	62.4 (1)	96.7 (5)	95.3 (4)	88.9 (6)	88.8 (2)	85.2 (3)
	6	59.6 (4)	85.7 (2)	86.5 (6)	89.3 (3)	85.8 (1)	82.5 (5)
T.D.N.	1	55.6 (2)	92.0 (1)	86.4 (3)	80.1 (5)	80.2 (4)	85.4 (6)
	2	54.6 (3)	102.3 (4)	82.1 (5)	86.1 (1)	81.6 (6)	69.2 (2)
	3	54.0 (6)	84.3 (3)	87.0 (1)	81.7 (2)	71.8 (5)	75.1 (4)
	4	53.1 (5)	77.7 (6)	84.9 (2)	81.8 (4)	79.0 (3)	75.4 (1)
	5	54.0 (1)	86.6 (5)	87.4 (4)	80.7 (6)	81.5 (2)	72.2 (3)
	6	54.2 (4)	82.1 (2)	76.3 (6)	81.8 (3)	79.7 (1)	72.7 (5)

TABLE 9.—ANALYSIS OF VARIANCE OF DATA IN TABLE 8†

Nutrient	Variance due to	D/F	Sums of squares§	Variance§	F
Dry matter	Ration	5	3,120.30	*	—
	Period	5	147.59	29.52	1.29
	Animal	5	129.29	25.86	1.13
	Error	20	445.96	22.97	—
Organic matter	Ration	5	3,392.70	*	—
	Period	5	160.74	32.15	1.51
	Animal	5	160.24	32.05	1.51
	Error	20	425.05	21.25	—
Nitrogen	Ration	5	1,356.29	*	—
	Period	5	481.49	96.29	3.99
	Animal	5	394.89	78.98	3.27
	Error	20	482.44	24.12	—
Ether extract extract	Ration	5	14,933.17	2,986.63	32.73
	Period	5	956.84	191.37	2.10
	Animal	5	368.69	73.74	†
	Error	20	1,825.05	91.25	—
Crude fibre	Ration	5	22,533.98	4,506.80	2.91
	Period	5	8,669.55	1,733.91	1.12
	Animal	5	15,633.87	3,126.77	2.02
	Error	20	31,004.26	1,550.21	—
N.F.E.	Ration	5	4,126.16	*	—
	Period	5	116.52	23.30	3.10
	Animal	5	46.34	9.27	1.24
	Error	20	150.08	7.50	—
T.D.N.	Ration	5	4,261.44	852.29	—
	Period	5	150.33	30.17	1.44
	Animal	5	142.54	28.51	1.36
	Error	20	419.74	20.99	—

† Nec. F for $N_1 = 5$, $N_2 = 20$ at P of 0.05 is 2.71 at P of 0.01 is 4.10.

* Obviously significant

† Variance for animal less than variance for error

§ Calculated to four decimal places, reported to two.

TABLE 10.—PER CENT DIGESTIBILITY OF GROSS ENERGY OF HAY AND OF BARLEY CALCULATED FROM RATIOMS WITH HAY ARRANGED BY RATIOMS, PERIODS AND ANIMALS.
(ANIMAL NUMBERS IN BRACKETS, COEFFICIENTS IN PER CENT)

Period	Hay	Digestibility of energy				
		Barley calculated from following rations with hay				
		H-3 B-1	H-3 B-2	H-3 B-3	H-3 B-4	H-3 B-4.7*
1	53.2 (2)	87.7 (1)	84.4 (3)	78.0 (5)	76.8 (4)	84.4 (6)
2	51.7 (3)	97.8 (4)	76.4 (5)	82.6 (1)	76.4 (6)	65.4 (2)
3	50.5 (6)	75.9 (3)	83.9 (1)	77.2 (2)	67.5 (5)	70.5 (4)
4	53.2 (5)	77.9 (6)	85.9 (2)	79.0 (4)	77.3 (3)	72.0 (1)
5	54.9 (1)	88.8 (5)	86.4 (4)	79.1 (6)	80.0 (2)	70.1 (3)
6	52.9 (4)	81.7 (2)	74.2 (6)	80.5 (3)	78.1 (1)	70.9 (5)

TABLE 11.—ANALYSIS OF VARIANCE OF DATA IN TABLE 10

Variance due to	D/F	Sums of squares†	Variance†	F	Nec. "F" at P = 0.05
Ration	5	4,014.74	*	*	2.60
Period	5	168.17	33.63	1.30	—
Animal	5	108.34	21.67	§	—
Error	20	518.17	25.91	—	—
Total	35	—	—	—	—

* Obviously significant.

§ Variance less than error variance.

† Calculated to four decimal places, reported to two.

BOOK REVIEW

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PASTURE STUDIES (XXIX). INVESTIGATIONS ON THE LIGNIN FRACTIONS OF PASTURE HERBAGE AND OF THE FECES OF RUMINANTS**I. THE LIGNIN FRACTION OF PASTURE HERBAGE¹**F. J. SOWDEN² AND W. A. DELONG³

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The importance of lignin in relation to the digestibility of pasture herbage often has been emphasized. For a number of years the Pasture Committee of Macdonald College has been conducting studies on the digestibility of pasture herbage (5, 6). These and other studies have shown that the usual methods of feedingstuffs analysis are not always satisfactory and it has been proposed that the determination of lignin and cellulose be substituted for the crude fibre determination (7). It has been stated that not only is lignin itself indigestible but also that it limits the digestibility of other nutrients (7, 19). However, different investigators have reported very different coefficients of digestibility for lignin from similar materials (9, 14, 2, 7). This is an indication that further work is necessary on methods of lignin determination before it can be used as a measure of the digestibility of a feed. Present methods of lignin determination are empirical and no unequivocal proof is available that the same material is isolated from forage as from feces. Correct interpretation of analytical results is therefore difficult. It appears that a study of the amount and nature of the lignin isolated by different methods of analysis would supply information that might aid in securing a more accurate picture of the metabolism of lignin. Accordingly the present investigation is divided into three parts (1) a study of the lignin of immature pasture herbage; (2) a study of the lignin isolated from feces of ruminants fed immature herbage; (3) a study of the products formed by the high pressure hydrogenation of forage and feces. This paper presents the results of the investigation of the first problem.

REVIEW OF LITERATURE

The structure of lignin is still unknown. Eastham *et al.* (8) have stated "the structural theories of Freudenberg and of Hibbert are still highly speculative in nature". It is by no means certain that there is only one lignin, for Freudenberg (10) has postulated that lignin may exist in wood in different degrees of condensation, varying from single units to complex aggregates. If this theory be true, lignin or its precursors might exist in young succulent tissue such as forage, in forms varying from the polyphenols to highly complex molecules. Wood lignin, which may be considered as typical of pure lignin, is nitrogen-free and usually contains over 12 per cent methoxyl (21).

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The ultraviolet absorption spectra of lignin and related compounds have been studied by a number of different investigators—Herzog and Hillmer (13); Hagglund and Klingsted (12); Stamm, Semb and Harris (20); Glading (11). These investigators have indicated that lignin is aromatic in nature, and also that it has a characteristic ultra-violet absorption spectrum, with an absorption maximum in the region of 2800 Å. This absorption band has been ascribed by Patterson and Hibbert (18) to freedom of the position meta to the side chain of the phenylpropane lignin building units, and it persists in spite of alteration of the lignin molecule by methylation, acetylation or treatment with phenol, glycol or thioglycolic acid (11). It is shown by lignin isolated in a wide variety of ways, e.g., by modifications of the H_2SO_4 method, by the Willstätter HCl method and by Freudenberg's cupra-ammonium method. It also has been demonstrated (20) that the presence of pentosans has no effect on the shape of the absorption curve nor on the extinction coefficients when the concentration of the lignin solution is corrected for pentosan impurity. The extinction coefficients were not greatly affected by the formation of lignin derivatives if a correction was made for the increase in molecular weight caused by their formation, unless the group added to the lignin molecule itself absorbed light in the region of 2800 Å (11).

It has been stated (17) that spectrographic methods are capable of identifying lignin in solution at a concentration of 2 mgm. per litre. Adams and Ledingham (1) used a spectroscopic method to estimate the amount of lignosulphonate in solution.

In the chlorinated products of the sulphuric acid lignin fractions, however, the 2800 Å maximum is not well defined in most cases, and is very poorly defined in the lignin fraction isolated from herbage. In spite of this lack of definition, it seemed that the absorption spectra of forage lignins might give some evidence of their purity if they were compared with the spectrum of wood lignin prepared and dissolved in the same manner.

In view of the uncertainty of the chemical nature of the lignin of young plants it seemed that useful information concerning the amount of lignin in forage could be secured only if the material isolated as lignin was carefully characterized. This was done by determining the content of nitrogen and methoxyl in the lignin fraction isolated and by measurements of the absorption spectra and the solubility of the chlorinated products in bisulphite solution.

MATERIALS

Materials were obtained from digestibility trials conducted at MacDonald College in 1942 and 1943 (Crampton and Jackson (6)). In the former year the forage samples were collected in early June, mid-July and mid-September. These periods were chosen since previous work (Crampton and Forshaw (5)) had indicated the mid-season herbage to be of lower digestibility than that of early or late seasonal growth. Timothy was the predominating species in the herbage throughout the season. In the 1942 season no attempt was made to estimate the percentage contribution of this species to the sward. At each of the three harvesting periods the forage was about 3-5 inches in height. In 1943 the herbage was cut at intervals

of about three weeks over the period June 1 to September 14. The latter season was abnormally wet and the forage remained succulent throughout the entire experimental period. A full description of the forage, including the species composition of the sward, is given by Crampton and Jackson (6).

PREPARATION OF MATERIAL AND METHODS OF ANALYSIS

The herbage samples collected in 1942 were dried at room temperature and ground to pass a 0.5 mm. sieve before analysis. Two procedures of lignin determination were employed, that of Manning and DeLong(16)—hereinafter referred to as the standard method—and that of Crampton and Maynard (7). In order to obtain consistent results by the latter method, it was found desirable to allow the lignin suspensions to settle for five minutes after boiling off the chloroform, and to filter rapidly. In 1943 the fresh herbage samples were extracted twice in a Waring Blender with an ether-water mixture, such treatment having been shown (McDougall and DeLong (15)) to permit the isolation of a less contaminated lignin fraction. This air-dried residue was treated subsequently as in the standard method.

The methoxyl and nitrogen contents of the lignin fractions isolated from each of the forage samples studied were determined, the object being to define, to some extent, the relative purity of these fractions. The methoxyl determinations were made by the method of Clark (3) using hydriodic acid prepared as he recommended (4). In the determination of nitrogen the lignin fractions were isolated in tared Gooch crucibles, dried at 105° C. and weighed, then digested by the Kjeldahl process using mercury as a catalyst. The solution was made up to 100 ml. and the nitrogen content of a suitable aliquot was determined using a micro-distillation apparatus. The methoxyl and nitrogen contents were corrected for the amount of ash in the lignin isolates.

Lignin fractions obtained from forage by both the standard and the Crampton-Maynard methods of isolation were examined spectrographically. These samples were chlorinated while in the freshly isolated, still moist, condition by placing the Gooch crucibles containing them in a desiccator, washing the air out of the latter by means of a current of chlorine prepared from KMnO_4 and HCl , closing the outlet from the desiccator and leaving the latter connected with the generator during the chlorination process. The time of contact of the moist lignin preparations with the gas varied from several hours to over-night, and three or four such chlorinations were required to effect the complete removal of the lignin from the lignin fractions isolated by the procedures employed. The chlorination products were separated from the residual material by washing with 2 per cent Na_2SO_3 solution. Chlorination and extraction were repeated until the leachate was colourless. The residue was then washed with water, dried at 105° C., weighed, ashed, and weighed again, the result being the amount of insoluble lignin fraction. Definite weights of feces and forage samples, 0.500 and 1.000 gm. for the standard and the Crampton-Maynard method respectively, were taken for isolation of the lignin fractions. From determinations previously made on the same samples the amounts of lignin fraction isolable by each of the two methods was known. Thus the weight of material soluble in 2 per cent Na_2SO_3 solution after chlorination was

determinable with a fair degree of accuracy. The sodium sulphite extracts were diluted with distilled water to a concentration suitable for spectrographic analysis.

A lignin fraction which would serve as a standard was prepared by the 72 per cent H_2SO_4 method from a sample of ground maple wood which had been extracted successively with ethanol-benzene (1 : 2) for 24 hours, 95 per cent ethanol for 24 hours, and hot water for 12 hours. A sulphite-soluble extract was prepared from the lignin fraction of wood meal in the manner already described for the forage and feces samples. It may be noted, at this point, that, while the lignin fraction obtained from maple wood dissolved readily and completely in 2 per cent Na_2SO_3 solution after a single chlorination, the fractions obtained from forage had to be chlorinated several times and extracted several times (as indicated above) before maximum solution had been attained.

The absorption data were determined using a large quartz spectrograph, Littrow mounting, with a 30° quartz prism, the spectral range from 2510 Å to 3450 Å being investigated. The photographic plates were of the Eastman No. 33 type, and the match points on these were determined visually. The extinction values of the match points were read directly from the settings of the rotating sector. Absorption curves were drawn plotting E as the ordinate against wave-length (in Å) as abscissa, the value of E at 2800 Å then being read from the graph. The values of the specific extinction coefficient (k) were calculated from the formula $T = e^{-cdk}$, when T = transmission, c = concentration and d = depth of solution. The values of k for wood lignin were found to be 38.9 and 39.3 for concentrations of sulphite-soluble material of 0.036 and 0.0068 gm./litre respectively; the average of these (39.1) is in fair agreement with that reported by Glading (11) for "native" lignin from maple wood, viz., 41.7. This "native" lignin preparation probably was a purer material than the lignin fraction obtained in the present investigation and hence might be expected to have a higher specific extinction coefficient. The k values for the forage and feces lignin fractions isolated by both the standard and the Crampton-Maynard procedures were calculated in the manner indicated above. On the assumption that the specific extinction of the sulphuric-acid lignin obtained from maple wood is typical of lignin preparations of high purity, the relative purity of the fractions obtained from forage may be estimated

from the formula $\frac{k \text{ of test solution}}{k \text{ of lignin solution}} \times 100$. This formula, of course, is,

valid only in the event that any non-lignin materials present have no absorption at 2800 Å, and have no effect on the absorption of lignin.

EXPERIMENTAL RESULTS AND DISCUSSION

The data on the amounts of lignin isolated from the 1942 forage samples and the percentages of methoxyl, nitrogen and ash found in these lignin fractions are summarized in Table 1. The percentages of ash are reported for the 1942 samples only. The analytical results reported here represent the averages of two or more determinations. The lignin, methoxyl and ash data are reported on the dry matter basis and have been corrected for their ash content.

TABLE 1.—NATURE OF LIGNIN ISOLATED FROM FORAGE MATERIAL
(Moisture-free, ash-free basis)

Cutting period	Method of isolation	Lignin	Methoxyl in lignin	Nitrogen in lignin	Ash in lignin
		%	%	%	%
1942					
1	Standard	6.10	5.50	2.77	12
2	Standard	6.84	4.10	3.83	8
3	Standard	5.77	2.82	5.31	6
1	Crampton-Maynard	11.14	3.37	5.38	17
2	Crampton-Maynard	8.20	3.62	3.94	18
3	Crampton-Maynard	11.10	2.19	4.42	12
1943					
2	Standard	8.83	4.93	5.47	—
3	Standard	8.23	4.07	5.58	—
4	Standard	7.78	3.17	6.64	—
5	Standard	7.81	3.01	7.06	—
6	Standard	6.41	3.00	7.80	—

Table 1 shows that the two methods isolate widely different quantities of material. The fact that the material separated by the Crampton-Maynard procedure in all instances contained less methoxyl and, with one exception, more nitrogen also, indicates that this fraction contains more non-lignin material than does that isolated by the standard method. The Crampton-Maynard procedure separates a greater amount of methoxyl from 100 gm. of dry matter in all instances, the difference for 1942 samples being 15, 4 and 50 per cent for the three harvests respectively. On the assumption that the only source of methoxyl in the lignin fractions isolated is lignin, these data suggest that, although impure, the Crampton-Maynard lignin fraction actually contains more of the lignin of the sample than does the fraction isolated by the standard method. Nevertheless, even on the basis of the assumption that the Crampton-Maynard fraction contains more lignin than does that obtained by the standard method, the former must contain considerable non-lignin material since it is larger than the latter by 82, 20 and 92 per cent.

It will be noted that in 1943 the percentage of lignin fraction in the forage decreased continuously throughout the season. This decrease may be related to weather conditions which, as had already been noted, were abnormally wet. The actual decrease in the lignin content of the forage probably was greater than the decrease in the amount of the lignin fraction isolated, since there was progressively greater contamination of the latter by nitrogenous substances. There was also a decrease in the methoxyl content of the lignin fractions isolated from forage samples 3 and 4 as compared to that of sample 2. There was, however, little change in the methoxyl content of the fractions obtained from samples 5 and 6 even though the nitrogen content of these fractions continued to increase. The increased nitrogen content of the lignin fraction from samples 3 and 4 may be accounted for by the increased proportion of clover in the sward. On the other hand, although sample 6 contained a lower percentage of clover than sample 4, the nitrogen content of the lignin fraction isolated from the former was greater

than that separated from the latter. There is, nevertheless, the suggestion that the presence in the sward of varying proportions of leguminous plants may influence the degree of contamination of the lignin fraction with nitrogenous compounds. This suggestion is supported by the fact that when the proportion of clover in the sward increased from 30 to 65 per cent the Crampton-Maynard lignin fraction doubled in amount, Crampton and Jackson (6). Since each cutting was made at approximately the same stage of growth as judged by height of forage and since there were no hot dry periods to promote rapid maturity, it appears that the species composition of the sward has a very marked effect on the quantity of lignin fraction isolated by the Crampton-Maynard procedure. Such observations as these indicate the desirability of conducting lignin studies on pure species of forage plants at least until such time as more definitive methods for the determination of lignin are available.

The data obtained for the percentage purity of the lignin fractions from the forage samples are presented in Table 2. The values for the relative purity of the sulphite solutions were obtained by the calculation described in the methods of analysis. The data on the relative purity of the lignin fractions were obtained by multiplying the percentage solubility of the lignin fraction by the relative percentage purity of the sulphite solution obtained from it. This procedure was adopted after it was observed that the specific extinction and the sulphite solubility of the lignin fractions bore an inverse relation to each other in the duplicate determinations (the tabulated values are averages).

There is a tendency for the inverse relationship of the specific extinction and the sulphite solubility to hold true for the various samples and hence for the relative purity to be fairly constant. For the 1943 samples the

TABLE 2.—RELATIVE PURITY OF LIGNIN FRACTIONS ISOLATED FROM PASTURE HERBAGE AS CALCULATED FROM VALUES FOR THE SPECIFIC EXTINCTION COEFFICIENT AS 2800 Å AND THE SOLUBILITY IN SODIUM SULPHITE SOLUTION: VALUES FOR LIGNIN FRACTIONS SIMILARLY ISOLATED FROM MAPLE WOOD USED AS THE STANDARD OF COMPARISON

Cutting period	Specific extinction coefficient (2800 Å)	Solubility in sulphite solution	Relative purity of solution	Relative purity of lignin fraction
		%	%	%
1942				
1 (S)*	20.2	83	52	43
1 C & M**	21.3	78	54	42
2 (S)	16.3	81	42	34
2 C & M	22.4	78	57	44
3 (S)	26.5	65	67	44
1943				
2 (S)	26.0	82	67	55
3 (S)	25.0	71	64	45
4 (S)	23.5	66	60	40
5 (S)	21.0	73	54	39
6 (S)	26	65	67	44

* (S)—Standard method.

** C & M—Crampton-Maynard method.

variation in the species composition of the herbage may obscure this; the relative purity is lower when there is a greater percentage of clover in the sward. It will be noted that the relative purity of the 1943 lignin fractions is in general higher than that of those isolated from 1942 samples. This may be attributed to the removal of soluble material from the 1943 samples before drying them. Since the relative purity of the Crampton-Maynard lignin fractions is the same as that of similar fractions obtained by the standard procedure, and since the amount of the former is considerably greater than that of the latter, the suggestion previously made (on the basis of the absolute amounts of methoxyl in these fractions) that the Crampton-Maynard fractions contain more of the lignin of the sample than do those obtained by the standard method is supported by the spectrographic date. There are at least three possible explanations of this difference: (a) the treatment previous to the isolation of the lignin fraction by the standard method may remove lignin; (b) the pre-treatment employed in the Crampton-Maynard procedure may fail to remove nitrogenous substances (such as tryptophane and tyrosine) giving absorption at 2800 Å, and, (c) the formaldehyde used in the latter procedure may react with the samples to give products absorbing light at 2800 Å.

Two of the striking characteristics of the lignin fractions isolated from herbaceous plant tissues at growth stages of increasing maturity are the very low methoxyl content of the material isolated from very young and succulent tissues, and the marked increase in this constituent of the lignin fraction as the maturity of the tissues increases. These features are usually considered to be due to a combination of two factors—contamination of the lignin fractions isolated from immature tissues, and increasing content of methoxyl per unit of lignin with increasing maturation. Beyond a relatively early stage in cellular development, however, it is difficult to visualize a mechanism for the methoxylation of lignin. It therefore appears probable that the contamination factor may be the more important. The spectrographic data lend support to this suggestion by emphasizing the fact that the amount of non-lignin material in the fractions isolated from succulent pasture forage is large.

Typical absorption curves obtained for lignin isolated by the standard method from the 1942 samples are shown in Figure 1. Lignin isolated by the Crampton-Maynard method and by the standard method from the 1943 samples gave similar curves and hence these are not presented. The curve obtained from a sample of wood lignin is shown for comparison.

These curves were drawn by plotting $E \frac{1\%}{1 \text{ cm.}}$ as the ordinate against wave length (in Å.) as the abscissa. The $E \frac{1\%}{1 \text{ cm.}}$ values were calculated from the extinction and concentration of the solutions using the relationship $E \frac{1\%}{1 \text{ cm.}} = \frac{E}{C}$, where C is the concentration of sulphite-soluble materials in gm./100 ml. The concentration of sulphite-soluble material was corrected for the non-lignin substances present on the assumption that the wood fraction consisted of lignin only and pure forage lignin would have the same absorption at 2800 Å. as wood lignin.

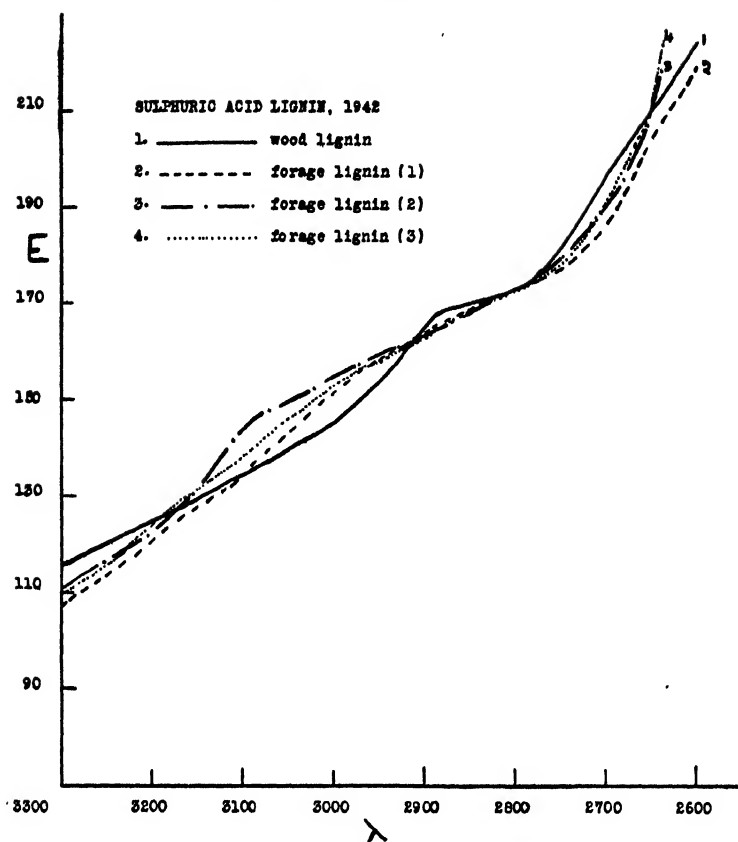


FIGURE 1. Absorption spectra of lignin solutions

The absorption curves for the forage lignin are generally similar to that obtained from wood lignin except that the break on the curve at about 2800 Å. shown by the latter is less pronounced or lacking in the former, but the similarity of the curves would indicate the presence of a material similar to wood lignin.

SUMMARY

The two methods of lignin determination that were studied were found to isolate widely different amounts of lignin from forage. The nitrogen and methoxyl values for the lignin fractions isolated indicated that these fractions differed also in their purity. It is possible that the Crampton-Maynard method isolated a less pure lignin than the standard method. It may recover more of the lignin of the sample, however.

Spectrographic data indicated that the lignin fractions isolated by either method were highly contaminated with non-lignin materials. The forage lignin contained large amounts of nitrogen and was low in methoxyl content (about one-third of the amount found in wood lignin).

It is also indicated that the ratio of clover to grasses in the immature herbage samples may influence the nature of the lignin fraction isolated.

In general the data here presented indicate that the current methods for determining lignin in young plant tissue are so inaccurate that conclusions respecting the digestibility of lignin based on the use of such analytical procedures are of questionable validity.

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PASTURE STUDIES (XXX). INVESTIGATIONS ON THE LIGNIN FRACTIONS OF PASTURE HERBAGE AND OF THE FECES OF RUMINANTS

II. THE LIGNIN FRACTION OF THE FECES OF RUMINANTS¹

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In the preceding paper of this series (4) the authors discussed the problem of the determination of the lignin content of immature pasture herbage. Existing data on the digestibility of lignin were also discussed. The fact that the coefficients of digestibility of lignin have been found to vary widely suggests that lignin procedures isolate a variable entity which may not have the same composition when derived from feces as when derived from forage. Accordingly it was decided to study the nature of the lignin fraction of feces. It is the purpose of this paper to present the results of these studies. The pertinent literature has been reviewed in an earlier paper (4).

EXPERIMENTAL MATERIALS AND METHODS

Materials

The feces samples were obtained from the same digestibility trials as the forage samples (4). In 1942 a steer was used as the experimental animal while in 1943 the trials were conducted with sheep. In 1942 the feed was cut daily with a lawn mower and fed immediately and in 1943 the forage was cut at tri-weekly intervals and artificially dried. The feces samples were collected for the same sample periods as the forage samples (4).

Preparation of Material and Methods of Analysis

Composite feces samples were collected over a 4-5 day period. The aliquot obtained from each 24-hour feces collection was stirred with an equal volume of 95 per cent ethanol and filtered with suction. This procedure was adopted to facilitate drying of the feces sample. Drying in air was employed since it was thought that oven-drying might cause condensation of labile materials and result in a high yield of a very impure lignin fraction (2). The air-dried samples were combined to form the composite sample which was used for analysis. This sample was ground to pass a 0.5 mm. sieve.

In 1943 aliquots of the feces voided in each 24 hours were taken over a 10-day period and kept in a frozen condition to form a composite sample for analysis. Since it had been shown previously (3) that lower yields of lignin were obtained if the water-soluble material of forage was removed prior to drying, an attempt was made to evolve a method of removing as much of the nitrogenous and other interfering materials as possible from feces before drying for treatment with 72 per cent sulphuric acid. The method used for this purpose in the case of the forage material (extraction in the Waring Blendor with cold ether-water) was not satisfactory for feces since it was very difficult to dry the resulting residue. Extraction in the Blendor with a 10 per cent CaCl_2 solution was more rapid but three extrac-

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TABLE 1.—EFFECT OF METHODS OF PREPARING SAMPLES FOR 72 PER CENT H_2SO_4 TREATMENT

Treatment	Lignin in feces per cent	N in extracted material	N in isolated lignin	N of extracted feces in lignin*
Oven dried		%	%	%
Standard	15.66	2.49	4.27	76
1 per cent HCl, ethanol-benzene	16.32	2.19	3.82	73
10 per cent $CaCl_2$, ethanol-benzene, 1 per cent HCl	15.33	1.97	3.29	70
Frozen and extracted before drying				
Ether-water, ethanol-benzene, 1 per cent HCl	10.53	1.11	2.32	76
Ether-water, 1 per cent HCl ethanol- benzene	12.36	1.59	3.06	75
10 per cent $CaCl_2$, ethanol-benzene, 1 per cent HCl	11.72	1.14	2.49	77
Boiling water, 1 per cent HCl, ethanol- benzene	10.17	1.25	2.69	76

Note—Extraction treatments are listed in the order in which they were applied.

* Represents the percentage of the nitrogen of the extracted material that remains in the isolated lignin.

tions were required to remove the maximum amount of soluble material. The $CaCl_2$ solution was used in the hope that it would remove mucoproteins and thus facilitate drying. It was found that disintegration of the material in the Blendor followed by treatment with boiling water and 1 per cent HCl (both for 3 hours in the proportion of 150 ml./gm. dry wt.) gave as low a yield of apparent lignin as the ether-water extraction, though the lignin isolated after hot water extraction was somewhat more contaminated with nitrogen. As the hot water modification of the method was found to be the most convenient of those tried, it was used for the preparation of 1943 samples 2-6. The percentage of the nitrogen of the extracted materials remaining in the isolated lignin was calculated from data on the weights of the extracted material and of the lignin isolated from it and the nitrogen contents of these materials. The treatments and the resulting data are shown in Table 1; the extractions were carried out in the order indicated.

It is obvious that oven-drying results in the isolation of a larger amount of apparent lignin which is more contaminated with nitrogen than is the case with samples that are dried at room temperature, after removing soluble material. The methods of extracting the forage sample gave somewhat similar results though reversing the order of extraction with 1 per cent HCl and ethanol benzene gave a larger yield of the lignin fraction; this was also true in the case of the oven-dried sample. Extraction in the cold with ether-water or 10 per cent $CaCl_2$, drying, then extracting with ethanol-benzene yielded the lignin fraction least contaminated with nitrogen. Since 70-77 per cent of the nitrogen of the extracted material remained in the isolated lignin it is apparent that nitrogen must be removed prior to the 72 per cent H_2SO_4 treatment if a relatively pure lignin fraction is to be isolated.

The pre-treatment involving extraction with boiling water was most convenient to use, since the dissolved materials could be removed from the resulting suspension by filtration. In the case of extraction in the cold with ether-water, or with 10 per cent CaCl_2 solution, the resulting suspension had to be centrifuged. For this reason the boiling water-extraction of the feces materials was used for the samples collected in the 1943 season.

RESULTS AND DISCUSSIONS

Table 2 shows the percentage of lignin in the feces samples and the methoxyl, nitrogen and ash contents of the isolated lignin. These results represent the average of two or more determinations. The lignin values are reported on a dry matter basis and all figures have been corrected for the ash content of the crude lignin isolated.

The data indicate that the Crampton-Maynard method isolates a larger amount of lignin material than does the standard method; however, the Crampton-Maynard lignin probably is less pure since it is lower in methoxyl and higher in nitrogen in each instance. The Crampton-Maynard method does isolate a greater amount of methoxyl from 100 gm. of dry material in each case, the differences being 38, 21 and 36 per cent for samples 1, 2 and 3, respectively; the total apparent lignin isolated is, however, 80, 48 and 54 per cent greater. This indicates that the Crampton-Maynard procedure actually isolates more of the lignin of the sample although the product is less pure. This conclusion is further supported by the spectrographic data reported below. The fact that the lignin fraction isolated is more contaminated with nitrogen is an indication that pepsin digestion is less effective in the removal of nitrogen than is hydrolysis with 1 per cent HCl.

It is noteworthy that the lignin isolated from the 1943 samples is lower in nitrogen and higher in methoxyl than that isolated from the 1942 samples, in spite of the fact that the forage fed had a higher percentage of clover and hence was presumably higher in nitrogen in 1943. This may be due to the difference in pre-treatment procedure. It is also possible that the sheep digested interfering nitrogenous materials.

TABLE 2.—NATURE OF LIGNIN ISOLATED FROM FECES MATERIALS, RESULTS ON MOISTURE-FREE, ASH-FREE BASIS

Period	Method of isolation	Lignin	Methoxyl in lignin	Nitrogen in lignin	Ash in lignin
		%	%	%	%
1942					
1	Standard	14.85	4.84	3.47	25
2	Standard	16.18	5.09	4.19	16
3	Standard	16.32	3.63	4.14	17
1	Crampton-Maynard	26.69	3.74	4.49	33
2	Crampton-Maynard	23.92	4.59	4.74	28
3	Crampton-Maynard	25.08	3.23	4.68	23
1943					
2	Standard	14.75	6.60	3.67	—
3	Standard	15.57	5.96	3.61	—
4	Standard	12.39	6.10	3.31	—
5	Standard	11.65	5.38	3.63	—
6	Standard	8.74	5.34	3.21	—

It is interesting to compare the nature of the lignin isolated from feces with that of the lignin from corresponding forage samples as reported in the previous paper (4). Table 3 shows the average methoxyl and nitrogen contents of the lignin isolated from the 1942 samples of forage and feces by the standard and the Crampton-Maynard methods and from the 1943 samples by the standard method. The lignin isolated from forage is usually higher in nitrogen and lower in methoxyl than that recovered from feces. This indicates that the lignin fraction isolated from forage is less pure. If this estimate of purity is valid, then lignin digestibility coefficients calculated from such data would be too low. The methoxyl data are in contrast to those reported by Bondi and Meyer (1), who found feces lignin to be lower in methoxyl than forage lignin. The theory that lignin is demethoxylated in its passage through the animal body is not supported by our data.

TABLE 3.—METHOXYL AND NITROGEN CONTENTS OF FECES AND FORAGE LIGNINS

Period	Method of isolation	Methoxyl, per cent		Nitrogen, per cent	
		Feces lignin	Forage lignin	Feces lignin	Forage lignin
1942	Standard	4.51	4.14	3.93	3.97
1942	Crampton and Maynard	3.85	3.06	4.64	4.58
1943	Standard	5.88	3.64	3.49	6.51

TABLE 4.—RELATIVE PURITY OF LIGNIN FRACTIONS ISOLATED FROM FECES AS CALCULATED FROM VALUES FOR THE SPECIFIC EXTINCTION COEFFICIENT AT 2800 Å AND THE SOLUBILITY IN SODIUM SULPHITE SOLUTION; VALUES FOR LIGNIN FRACTIONS SIMILARLY ISOLATED FROM MAPLE WOOD USED AS THE STANDARD OF COMPARISON

Cutting period	Specific extinction coefficient (2800 Å)	Solubility in sulphite solution	Relative purity of solution	Relative purity of lignin fraction
		%	%	%
1942				
1 (S)*	23.8	67	61	41
1 C & M**	20.7	81	53	43
2 (S)	18.1	77	46	35
2 C & M	23.7	73	60	44
3 (S)	23.9	68	61	41
1943				
2 (S)	30.1	74	77	57
3 (S)	29.8	75	76	57
4 (S)	33.5	65	86	56
5 (S)	33.9	40	87	35
6 (S)	36.4	45	92	42

* (S)—Standard method.

** C & M—Crampton-Maynard method.

SPECTROGRAPHIC RESULTS

The spectrographic studies on the feces lignin were carried out in the same manner as those on the forage lignin.

The data obtained for the percentage purity of the lignin fractions isolated from the feces samples are given in Table 4. The values for the relative purity of the lignin solutions were calculated from the formula
$$\frac{\text{extinction of lignin test solution}}{\text{extinction of wood lignin solution}} \times 100.$$
 The relative purity of the lignin fractions was calculated by multiplying the relative percentage purity of the lignin solution by its percentage solubility in sulphite.

It is noteworthy that, as judged by the spectrographic data, the Crampton-Maynard lignin fraction is as pure as the material isolated by the standard method. This may indicate that the former method actually isolates more of the lignin from the sample than does the latter. It is probable that although the fraction isolated is not completely soluble in sodium sulphite, all or most of the true lignin is removed, since there is an inverse relation between the extinction and sulphite solubility. Where the solubility in sulphite is low the extinction is high. The data indicate that the lignin isolated by either method is about 50 per cent pure.

Absorption curves were drawn, plotting $E \frac{1\%}{1 \text{ cm.}}$ as the ordinate against wave length (in Angstrom units) as the abscissa. The concentration of the sulphite-soluble material was corrected for non-lignin substance present on the assumption that feces lignin would show the same absorption characteristics as wood lignin. Absorption curves for lignin isolated from the 1942 samples by the standard method are shown in Figure 1. The absorption of a sample of wood lignin is shown for comparison. Lignin isolated from the 1943 samples and by the Crampton-Maynard method from the 1942 samples showed similar light absorption properties. The curves are similar to that of wood lignin and would therefore indicate the presence of a lignin-like material.

It has been shown in this and in the preceding paper that the lignin fractions isolated by current methods from either grass or feces may be highly contaminated by extraneous compounds, and may contain considerable percentages of nitrogen. The lignin isolated by the methods employed does not seem to be radically different in its chemical nature although certain pre-treatments may lower the content of nitrogen. However, the amount of apparent lignin may vary rather widely. It is therefore understandable that variations in reported digestibility coefficients are so great. It is obvious that a great deal of work remains to be done on the chemistry of the lignin of herbage and feces before any accurate picture of its metabolism can be obtained.

SUMMARY

The nature of lignin isolated by different methods from the feces of ruminants fed immature pasture herbage has been studied.

Both the pre-treatment and the method itself have great influence on the amount and nature of the fractions isolated as lignin. Spectrographic data indicated that these lignin fractions were about 50 per cent pure

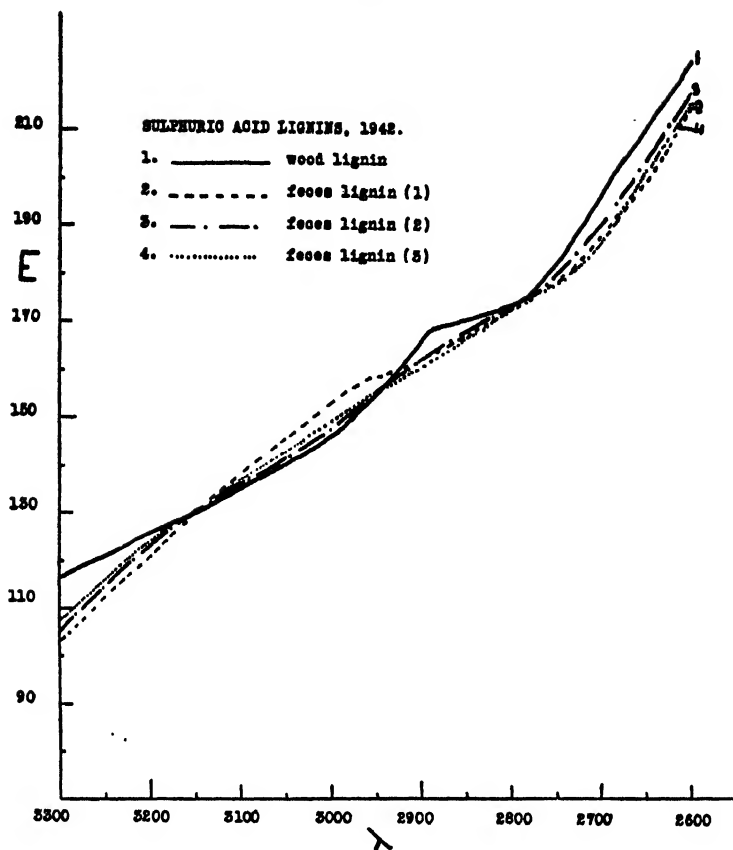


FIGURE 1. Absorption spectra of lignin solutions

relative to wood lignin. They contained relatively large amounts of nitrogenous material and were low in methoxyl. However, the data seemed to show that lignin was not demethoxylated in its passage through the animal body.

In view of the relatively low purity of the lignin fractions isolated by the methods studied, it is suggested that more intensive studies of the chemistry of grasses and of feces are required before the estimation of lignin will furnish an accurate index of the digestibility of herbage.

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SOME FACTORS AFFECTING APPLE YIELDS IN THE OKANAGAN VALLEY

VI. CONTENTS OF N, P AND K IN THE TERMINAL SHOOTS¹

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In a previous paper in this series (25), the P, K and Ca contents of the soil were discussed. In this present paper the information obtained from analysis of the terminal shoots of apple trees is presented. Data obtained from leaf analyses will be presented in subsequent papers.

Prior to the start of this investigation (1937), terminal and twig analysis had been used to a considerable extent as a general measure of the quantities of certain nutrients absorbed by the roots. The results obtained by other workers gave encouragement to the hope by the author that the nutrient status of fruit trees could be readily determined by analysis of terminal shoots. At the present time, indications are that leaf analysis offers more promise than does shoot analysis. This investigation, however, has produced considerable information of value with regard to terminal shoots, their N, P and K contents, and the effects of certain factors on these contents.

REVIEW OF LITERATURE

Variation Within Shoot

In studies on the nutrient contents of the terminal shoots of fruit trees, considerable variation has been found in the concentrations of the nutrients from point to point within the shoot. In 1923, Harvey (9) reported finding decreasing amounts of N from the tip toward the base of the shoot. In 1931, Cullinan (4) obtained similar results. In 1935, Warne and Wallace (21) found a lower N content in the bark than in the wood of terminal shoots. Similar results were reported later by other writers (28). In 1936, Waltman (18) found that soluble N increased from the base to the tip of the shoot, but soluble P did not. In 1941, Davidson (5) recommended using the tip five-inch portions of apple shoots for analysis, rather than the whole shoots.

Seasonal Variations

In 1923, Harvey (9) found that in all parts of an apple shoot the N content, expressed in per cent of dry weight, decreased rapidly from June 2 to August 2, but showed little further change to September 11. In 1927, Thomas (13) reported that the per cent N decreased very rapidly in new shoots until full bloom, then less rapidly until fall, when it tended to increase again somewhat. Total N per shoot did not follow the same curve as did per cent N. Somewhat similar results were obtained in 1931 by Sullivan and Cullinan (12) with the apple and by Mulay (10) with the pear, and in 1941 by Williams (28) with the peach. In 1938, Vaidya (15) reported that in the bark of apple shoots, P decreased from June to September, then

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remained fairly constant until April. The K content, however, decreased from June to December. In the wood of shoots, both P and K decreased from June to September, rose slightly to December, then remained constant until April. In 1948, Beattie (1) found that shoot weight increased from June to September then held steady, while the N content of the shoots decreased rapidly till August then increased slightly. The total N per shoot, however, increased during the season. There appears to be general agreement that the N, P and K contents of the shoot tend to decrease rapidly early in the season, and to be at or near a minimum just prior to harvest.

Effects of Fertilizers

In 1927, Thomas (14) found increased amounts of N in apple shoots following application of nitrate of soda. Similar effects of N fertilizers have since been reported by a number of investigators (3, 11, 27, 28). In 1930, Davis (6) reported that when insufficient amounts of N and K were supplied to apple trees growing in pots, their contents in the shoots were reduced. Wallace (16) obtained similar results with P, K and Mg, and Waltman (19) with N and P. In 1933, Wallace and Proebsting (17) reported that K deficiencies were clearly reflected in the K contents of apple and plum shoots. In 1941, Davidson (5) recommended the use of analysis of extracts of green twigs for the purpose of determining nutrient deficiencies. There appears to have been general agreement that the higher the content of a nutrient element in the soil, the higher is its content in fruit tree shoots.

PROCEDURE

As reported in the first paper in this series (23), 400 mature McIntosh apple trees were selected in 1937 in grower-owned orchards in the Okanagan Valley. Most of these trees were selected in groups or "plots" of five, with one or more such plots in each orchard. Records of tree vigour, yield, and "profitable yield" were obtained over a six-year period (1937-1942) on 290 of these trees, in 73 plots (23). The "profitable" fruits included only those with more than 20 per cent of solid red colour and within the size range of $2\frac{1}{4}$ to $3\frac{1}{8}$ inches in diameter.

Each of the 73 groups or "plots" of trees was treated by the grower in accordance with his own orchard practices. For the most part, uniformity was maintained in the fertilizer and other cultural operations from year to year in any one plot. Sick trees, or trees on which the cultural operations were varied too much during the six-year period, were eliminated from the investigation.

Soil samples were obtained from each plot in the spring of 1940, at depths of 0 to 8, 8 to 24, and 24 to 60 inches. Where gravel and coarse sand mixtures were encountered above 60 inches, samples were not taken to the full depth. The procedures used and some of the results obtained have already been reported (24, 25).

Terminal shoot samples were taken in the fall of 1939 and again in the fall of 1940. The purpose of taking these samples for two years in succession was to cover both an "on" year and an "off" year with trees bearing biennially. The time of sampling was the last week of August and the

first week of September. As already noted in the "Review of Literature", this was the time when changes in nutrient content of the shoots had been found to be at or close to a minimum. Moreover, the N, P and K contents appeared to be at or close to minimum values just prior to harvest. As a check on results obtained by other workers, samples of shoots were selected periodically from six trees in 1938, four trees in 1939, and four trees in 1941. The data obtained from this will be presented below under "Results".

The procedure used in sampling the shoots was as follows: Select 10 terminal shoots growing outward and upward at an angle around the lower perimeter of the tree. Repeat this around the upper perimeter, to obtain a total of 20 shoots. Remove and discard the leaves, and cut off the tip 5 cm. portion of each shoot for analysis.

The main reason for selecting the tip 5 cm. portion only for analysis, rather than the full length of the current shoots, was to obtain a more constant proportion of meristematic tissue to total tissue. As already noted under "Review of Literature", highest percentages of N, P and K are found toward the tip of the shoot, in which part the proportion of meristematic tissue is also the highest. Where the shoots vary greatly in length, as occurred in this investigation, selection of the whole shoot causes a much wider variation in proportion of meristematic tissue to total tissue.

The shoot samples were allowed to air dry, and were then washed in hot 3 per cent HCl to remove any adhering arsenical spray residues. They were dried to constant weight, weighed, and ground in a hand mill. Total N was determined (on only one or two trees in each plot) by a modification of the Kjeldahl-Gunning-Arnold procedure. For P and K analysis, an aliquot of dried material was wet oxidized with nitric acid and perchloric acid, by a modification of the procedure developed by Gerritz (7). Analyses for P and K were made as described in a previous paper in this series (25). Results were expressed in terms of parts per million of the dry weight of the shoot material.

Statistical analyses have been made of the data obtained, by methods already explained (23). Total yields were adjusted in turn for differences in size of tree, tree vigour, and biennial bearing, and profitable yields for differences in size of tree. These adjusted yields were then correlated with the two-year average N, P and K contents of the shoots. The biennial index (23) of each of N, P and K was calculated, and these in turn were correlated with one another and with the biennial indices for yield and terminal growth. Scatter diagram studies were made of each correlation.

RESULTS

Yields, tree vigour and other tree data have already been reported (23), as have also the soil analysis data (25). The records on shoot weights and N, P and K contents are so voluminous that no attempt will be made to present them in full in this paper. It will be considered sufficient to note the seasonal trends, the coefficients of correlation, and the special fertilizer plot data.

Seasonal Trends

Changes that occurred in weight of the tip 5 cm. of shoots during 1938, 1939 and 1941 are illustrated in Figure 1. The weight increased until November in 1938 and October in 1939. Beattie (1) in New York reported that shoot weight of apples increased until September only.

Changes in the contents of N, P and K are shown in Figures 2, 3 and 4, respectively. It will be seen that in 1938 the N content dropped rapidly to July, then rose slightly to December. In 1939 and 1941, the P content

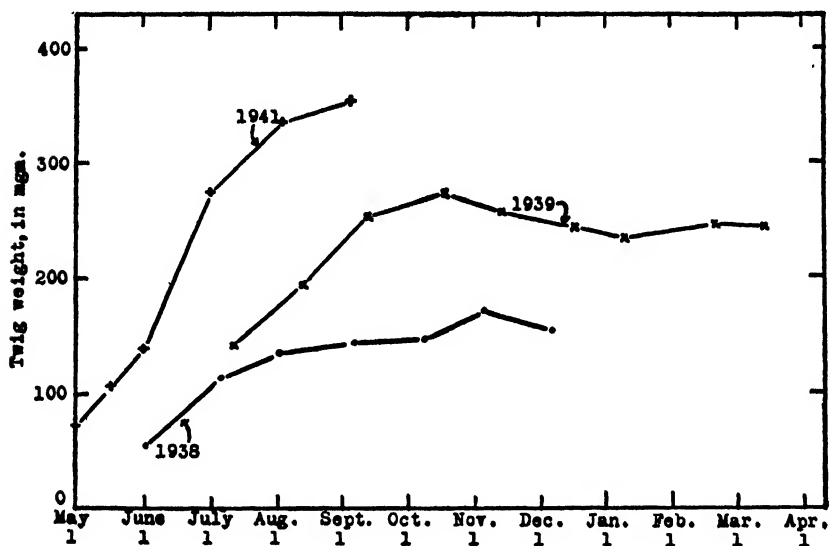


FIGURE 1. Trend of shoot weight during the growing season.

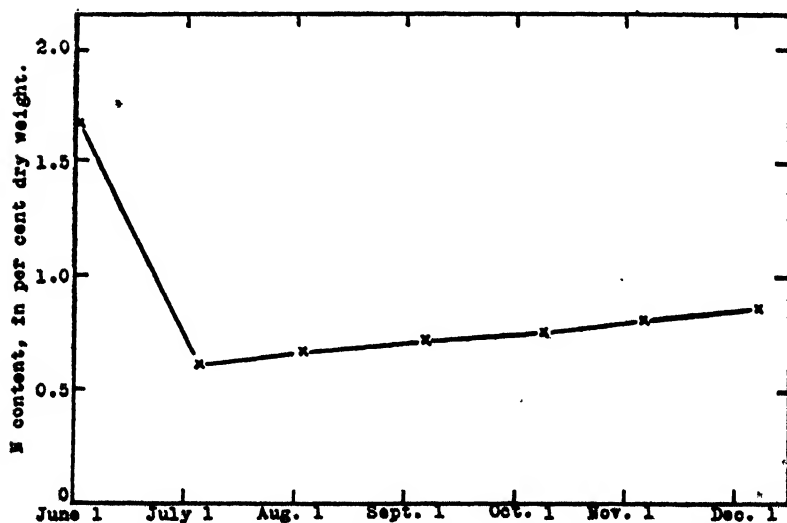


FIGURE 2. Trend of N content of shoots during the growing season.

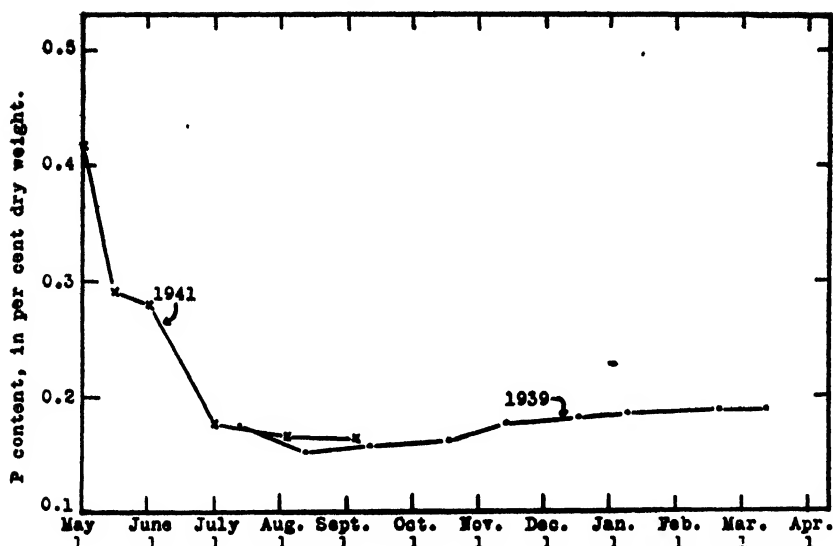


FIGURE 3. Trend of P content of shoots during the growing season.

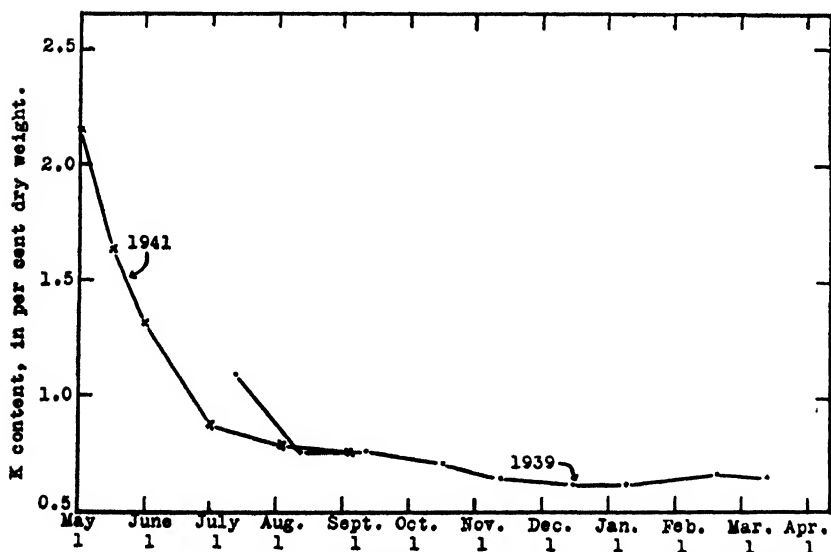


FIGURE 4. Trend of K content of shoots during the growing season.

dropped rapidly to July, less rapidly to August, then rose slightly to March while the K content dropped rapidly to July, more slowly to December, then rose again slightly over winter. In all cases, the most rapid changes occurred early in the season, while the shoots were still making their linear growth. The trend for N was similar to that reported by Thomas (13), and the P and K trends were similar to those reported by Vaidya (15). At the time of the main sampling—just prior to harvest—changes in N, P and K contents were slight.

Range of Values

The range of values obtained is important in the mechanics of both analysis and interpretation. Other things being equal, the greater the range in values the less difficulty arises from errors of chemical procedure, and the more readily can the data obtained be allocated to their class ranges. It is easier, for example, to divide the K content of a tissue into classes where it varies by several hundred per cent from sample to sample than where it varies by only 50 per cent or less.

The range of values obtained with N, P and K is summarized in Table 1. It will be seen that in all cases the extreme values obtained were not far apart; and this in spite of the fact that the trees sampled represented a very wide range in soil type and fertilizer treatment. This constitutes a distinct weakness in the use of shoot analysis as a means of diagnosing the nutrient status of fruit trees.

TABLE 1.—RANGES OF N, P AND K CONTENTS OF THE SHOOTS

Measurement	Year	Minimum	Maximum	Mean
N content, in per cent dry weight	1939	0.503	0.988	0.712
	1940	0.587	1.076	0.818
P content, in per cent dry weight	1939	0.132	0.211	0.170
	1940	0.136	0.203	0.166
K content, in per cent dry weight	1939	0.550	0.830	0.692
	1940	0.500	0.820	0.642

Fertilizer Plots

Included in the 73 plots of McIntosh trees were three series of fertilizer plots. Yields and soils data from these plots have already been reported (26). In each series, the fertilizer treatments that had been applied over a period of years included a check plot receiving no fertilizer, a plot receiving nitrogenous fertilizer only, a plot receiving nitrogen plus phosphate, and a plot receiving nitrogen, phosphate and potash. The average shoot analyses of the trees in these plots are shown in Table 2. By way of comparison, there are also shown the P and K contents of the surface soil (26).

It will be noted from Table 2 that the N, P and K contents of the shoots showed little if any effect of fertilizer application. This is certainly not encouraging in so far as the use of shoots to diagnose the nutrient status of the trees is concerned. As noted in the "Review of Literature", other investigators have obtained excellent results in this regard, the content of an element in the shoots having been almost invariably increased by feeding this element to the roots.

An examination of the scatter diagram charts of the P and K contents of the shoots, from the whole 290 trees studied, indicates the possibility that the shoots reflect the nutrient status of the soil more accurately under conditions of nutrient deficiency than under conditions of nutrient sufficiency. In other words, differences in luxury consumption do not appear to be reflected accurately in shoots collected just prior to harvest.

TABLE 2.—FERTILIZER PLOT DATA

Element	Orchard	Year	Plot treatment			
			O	N	NP	NPK
N in shoots (%)	Barnard	1939	0.73*	0.69	0.60	0.62
		1940	0.81	0.84	0.76	0.75
	Butler	1939	0.75	0.81	0.87	0.76
		1940	0.76	0.80	0.81	0.78
	Willits	1939	0.73	0.84	0.84	—
		1940	0.87	0.95	0.94	—
P in shoots (%)	Barnard	1939	0.17	0.17	0.17	0.17
		1940	0.17	0.15	0.15	0.16
	Butler	1939	0.18	0.17	0.21	0.16
		1940	0.18	0.16	0.16	0.18
	Willits	1939	0.16	0.17	0.18	0.17
		1940	0.18	0.18	0.19	0.18
K in shoots (%)	Barnard	1939	0.77	0.72	0.75	0.78
		1940	0.66	0.64	0.71	0.70
	Butler	1939	0.73	0.67	0.71	0.72
		1940	0.61	0.63	0.64	0.67
	Willits	1939	0.70	0.69	0.67	0.70
		1940	0.65	0.66	0.62	0.60
P in soil (p.p.m.)	Barnard	1940	0.8	3.3	11.9	10.2
	Butler	1940	2.0	1.6	8.4	8.2
	Willits	1940	1.4	0.8	16.4	—
K in soil (p.p.m.)	Barnard	1940	69	105	81	132
	Butler	1940	74	80	80	110
	Willits	1940	68	64	87	—

* Each figure for % N has been obtained from only one tree in the plot. Each figure for % P or K is an average of the values obtained from three to five trees in the plot. Each figure for p.p.m. P or K in the soil is from a composite 0-8 inch sample obtained from 10 locations in the plot.

As noted in a previous paper (26), no yield response from P or K has yet been obtained in these three series of fertilizer plots, indicating that there is as yet no proof of any deficiency of these elements in the three orchards represented. This, then, may explain the lack of response in the P or K content of the shoots.

It will be noted that application of phosphate has been accompanied by a marked increase in the available P content of the soil, but that application of potash has induced only a small increase in available K. These soils were already rich in available K without fertilizer treatment.

Effects of Biennial Bearing

Many of the 290 trees were addicted to biennial bearing, i.e., a heavy crop one year followed by a light crop or no crop the following year. The total yield per tree was used in determining the degree of biennial bearing. From the yields of each of two consecutive years was calculated the "biennial bearing index" of each tree as follows:

$$\text{Biennial bearing index} = \frac{100 (\text{difference between two yields})}{\text{sum of two yields}}$$

Complete annual bearing gave an index of zero, and complete biennial bearing an index of ± 100 . When the yield of the second year was the

heavier, the index bore a plus sign; and when the yield of the first year was the heavier, it bore a minus sign. In computing averages over a period of years, these signs were ignored. This same procedure was used for calculating the biennial indices for terminal length, shoot weight, % N, % P, and % K in the shoots.

In order to determine the effects of biennial bearing on the shoot weight and its N, P and K contents, correlations were calculated among the biennial indices concerned. Those results of interest are presented in Table 3. An examination of the coefficients of correlation reveals that when a tree is bearing biennially, the following situation is found in the "on" year as compared with the "off" year:

Higher yield
Longer terminal shoots
Thinner terminal shoots (near the tips)
Higher N content of shoots
Lower P content of shoots
Lower K content of shoots

Recent work by the author (not yet reported) indicates similar effects of biennial bearing on the leaves as are reported here on the shoots.

The effects of biennial bearing on length and diameter of terminal shoots have already been discussed (22, 23). The lower P and K contents in the shoot and leaf tissues during the "on" year can be attributed primarily to heavier utilization of these elements by the developing fruits. Why the N content is higher in the "on" year is more difficult to explain. Wander (20) in Ohio has found more N in apple leaves during the "off" year, but Cain and Boynton (2) in New York have reported finding more N in the "on" year, and more P and K in the "off" year.

TABLE 3.—CORRELATIONS BETWEEN 1939-40 BIENNIAL INDICES

Two sets of data correlated		Coefficient of correlation
Biennial bearing index	Biennial terminal length index	+ 0.634**
Biennial bearing index	Biennial shoot weight index	- 0.625**
Biennial terminal length index	Biennial shoot weight index	- 0.561**
Biennial N index	Biennial bearing index	+ 0.330**
Biennial N index	Biennial terminal length index	+ 0.682**
Biennial N index	Biennial shoot weight index	- 0.225*
Biennial P index	Biennial bearing index	- 0.231**
Biennial P index	Biennial terminal length index	+ 0.023
Biennial P index	Biennial shoot weight index	+ 0.154*
Biennial K index	Biennial bearing index	- 0.316**
Biennial K index	Biennial terminal length index	- 0.379**
Biennial K index	Biennial shoot weight index	+ 0.459**
Biennial N index	Biennial P index	+ 0.601**
Biennial N index	Biennial K index	- 0.322**
Biennial P index	Biennial K index	+ 0.038

** Coefficient of correlation highly significant (odds greater than 99 : 1). * Coefficient significant (odds between 19 : 1 and 99 : 1). Coefficients not marked are considered non-significant (odds less than 19 : 1).

These results have an important bearing on the selection of plant material for evaluation of its nutrient status. The possibility of variations occurring in the nutrient content of fruit tree shoots or leaves, as a result of differences in cropping, indicates the advisability of selecting plant material from trees cropping to the same degree. The question arises as to whether sampling should be done on trees heavy in crop or light in crop. If it is considered desirable to determine each nutrient when it is present in least sufficiency, then the above data suggests the "off" year for N and the "on" year for P and K. The next best approach would appear to be to take composite samples representing an average degree of cropping in each case, and to set up standards of sufficiency on this basis.

Correlations with P and K Content of Soil

Correlations between the P and K contents of the shoots and the P and K contents of the soil are summarized in Table 4. Good positive correlations were obtained between the available P content of the soil and the P content of the shoots. This is rather surprising, in view of the apparent lack of such a relationship in the three series of fertilizer plots discussed above. The correlations between the available K content of the soil and the K content of the shoots were positive but non-significant. A possible explanation for this difference between P and K can be drawn from the assumption made above that the P or K content of the shoot bears a closer relationship to that in the soil when the supply of available P or K in the soil is low. On the whole, the soils in the 73 plots appear to average much lower in P content (relative to sufficiency) than in K content. It would be anticipated, therefore, that the coefficients of correlation between the P contents of the soil and the shoots would be greater than those between the K contents of the soil and the shoots.

Effects on Tree Vigour and Yield

To determine the relationships between N, P and K contents of the shoots on the one hand and the vigour and yield on the other hand, the data were averaged for two or more years and were then correlated. Only two years of records (1939 and 1940) were averaged with shoot weight and N, P and K contents, while four to six years of data were averaged with terminal length, biennial bearing index and yield. The correlations obtained are presented in Table 5.

TABLE 4.—CORRELATIONS BETWEEN P AND K CONTENTS
OF SHOOTS AND SOIL

Two sets of data correlated		Coefficient of correlation
P in soil 0- 8 inches	P in shoots†	+ 0.289*
P in soil 8-60 inches	P in shoots	+ 0.311**
P in soil 0-60 inches	P in shoots	+ 0.319**
K in soil 0- 8 inches	K in shoots	+ 0.185
K in soil 8-60 inches	K in shoots	+ 0.041
K in soil 0-60 inches	K in shoots	+ 0.196

** Highly significant (odds greater than 99 : 1).

* Significant (odds between 19 : 1 and 99 : 1).

† Average of two years, 1939 and 1940.

The coefficients of correlation in Table 5 are disappointingly low in comparison with those in Table 3. Outstanding are the strong positive correlations between the N and P contents, between the P content and the shoot weight, and between the P content and terminal length. It appears that either N and P occur together naturally in these soils, or else they occur together in the shoots because of their close physiological relationship.

Of special interest also is the significant positive correlation between the N content and the length of terminal shoots. The question arises, then, as to whether average terminal length can safely be used as a measure of the N status of the tree. As noted elsewhere (26), the average terminal length of fruit trees is now being used in the Okanagan Valley as a basis for recommendations with respect to nitrogenous fertilizers. A more accurate procedure would undoubtedly be to determine the N content of the tree tissue. While one shoot analysis is being made, however, a large number of terminal lengths can be measured. So far, there has been little evidence in the Okanagan Valley of effects of soil nutrients other than N on tree vigour. The use of terminal length as a measure of N requirements would no doubt be less reliable in other areas, suffering from deficiencies of soil moisture or nutrients other than N.

TABLE 5.—CORRELATIONS BETWEEN NUTRIENT CONTENTS OF SHOOTS AND TREE PERFORMANCE

Two sets of data correlated		Coefficient of correlation
Average N content†	Average P content	+ 0.410**
Average N content	Average K content	— 0.039
Average P content	Average K content	— 0.037
Average N content	Average shoot weight	— 0.065
Average N content	Average terminal length	+ 0.280*
Average N content	Average biennial bearing index	— 0.098
Average N content	Total yield	+ 0.262*
Average N content	Profitable yield	+ 0.171
Average P content	Average shoot weight	+ 0.300**
Average P content	Average terminal length	+ 0.126*
Average P content	Average biennial bearing index	+ 0.098
Average P content	Total yield	+ 0.049
Average P content	Profitable yield	— 0.044
Average K content	Average shoot weight	+ 0.103
Average K content	Average terminal length	+ 0.165
Average K content	Average biennial bearing index	+ 0.149
Average K content	Total yield	— 0.129
Average K content	Profitable yield	— 0.152

** Highly significant (odds greater than 99 : 1).

* Significant (odds between 19 : 1 and 99 : 1).

† Averages of the two years, 1939 and 1940, were used with the shoot weight and the N, P and K contents. Six-year averages were used with terminal length, biennial bearing index and yield.

No significant relationship is revealed in Table 5 between the N, P or K content and the biennial bearing index. This was rather a surprise, as observation appears to indicate lessened biennial bearing with greater vigour. The fact that neither the N content of the shoots nor terminal

length has been correlated closely with the degree of biennial bearing can be attributed primarily to meteorological conditions (23). Among these conditions are late spring frosts and (to a lesser extent) winter killing of fruit buds. It appears also that growth conditions at time of fruit bud initiation are partly responsible.

With one exception, the correlations with yield (in Table 5) were not statistically significant. A significant positive correlation was obtained between the N content of the shoots and yield. This confirms the close relationship previously reported (23) between terminal length and yield. There were, however, very low correlations between P content and yield, and negative (non-significant) correlations between K content and yield. It appears from this that there have been no general deficiencies of P and K encountered in this investigation. If such deficiencies have occurred, they have been effectively masked by other factors. It is difficult to explain the negative correlations between K content of the shoots and yield.

DISCUSSION

The data presented in this paper give little encouragement for the use of shoot analysis as a means of determining the nutrient status of fruit trees. It is quite possible that the method could be used satisfactorily where marked deficiencies of certain elements occur; but it has not proved reliable in this investigation as a measure of the amounts of available P or K in soil containing more than the minimum requirements. From this viewpoint, both soil analysis and leaf analysis have proved more satisfactory. Whatever method is used, it should be one that provides ready measurement not only of deficiency of an element, but also of the degree of sufficiency.

Stress needs to be laid on the proper selection of tree tissue for analysis. It appears to be generally conceded (5, 8) that for routine analysis it is important always to select only one type of tissue, and to do so at only one part of the season. In addition to this, however, it appears desirable to collect samples only from trees showing a certain degree of cropping. To determine P and K deficiencies, sampling during the heavy crop year shows good promise. To determine the status of nutrients other than N, it also appears desirable to standardize the type of tree used, with regard to degree of vigour.

The use of shoot analysis has not revealed any deficiencies of P or K in the soils of the Okanagan Valley. The possibility that such deficiencies may actually exist has, however, been suggested by soil analysis (25). The final proof of deficiency of P or K is considered to be plant response to fertilizer application, and selected orchards showing low P and K contents of the soil are now being tested for such response by the field plot technique.

SUMMARY

Tree vigour and yield were recorded for six years on 290 mature McIntosh trees in the Okanagan Valley. Soil samples were taken at depths of 0-8, 8-24 and 24-60 inches. During two successive years, samples

of terminal shoots were collected prior to harvest, and the tip 5 cm. portions were analysed for total N, P and K contents. Shoot samples were also collected at different periods during the season.

During the growing season, shoot weight increased until after harvest; the N content dropped rapidly till July, then rose somewhat; the P content dropped rapidly till August, then rose slightly; and the K content dropped rapidly till July then more slowly till December.

The ranges of N, P and K values obtained were not large, in spite of wide variations in soil type and in fertilizer treatment.

The application of N, P or K fertilizer had little effect on the N, P or K contents of the shoots. Indications were that the N, P and K contents of the shoots reflect variations in the respective nutrients in the soil less accurately when they are present in sufficiency than when they are present in deficient amounts.

Biennial bearing of the trees was found to affect the N, P and K contents of the shoots in any one year. During the "on" year the terminal shoots grew longer but were narrower near the tips, the N content was higher, and the P and K contents were lower, then during the "off" year.

Correlations between the available P content of the soil and P in the shoots were positive and significant, and between the available K content of the soil and K in the shoots positive but non-significant.

Strong positive correlations were obtained between the N and P contents of the shoots, between the N content and terminal length, between the P content and terminal length, and between the P content and shoot weight. Correlations between the average N, P and K contents and the degree of biennial bearing were very low and non-significant.

Correlations between the N content of the shoots and the yield were positive and significant. Those between the P and K contents and the yield, however, were not statistically significant.

It is concluded that the use of shoot analysis has not revealed any deficiencies of P or K in Okanagan Valley apple orchards; also that shoot analysis does not show great promise as a means of diagnosing the nutrient status of fruit trees.

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THE PERFORMANCE OF SOUTHERN STRAINS OF BROME GRASS IN WESTERN CANADA¹

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Brome grass is the most important cultivated forage grass in Western Canada. Following the period of extensive settlement of the Prairie Provinces after 1900 brome grass became widely used for hay and pasture purposes and frequently escaped from cultivation to form a common grass cover on road sides, abandoned lands, and other disturbed areas. In recent years a keen interest has developed in certain districts within the general adaptation area in the production of brome grass seed. Of a total Canadian production of 7,594,000 pounds in 1947 the Provinces of Manitoba, Saskatchewan, and Alberta produced 7,500,000 pounds⁴. The greater part of this seed has been exported to the United States.

In 1943, Newell and Keim (10) indicated that local Nebraska strains of brome grass which they termed "southern" strains were much superior to introduced or "northern" strains under conditions in Nebraska. The marked superiority of southern strains subsequently found in several adjoining states and the dependence of Canadian seed production on markets in the United States made it imperative to study the performance of southern strains in Western Canada. Since 1938, comparative trials of northern and southern brome grass strains have been conducted at eight Dominion Experimental Stations in Western Canada and at the Dominion Forage Crops Laboratory, Saskatoon, Sask. This paper summarizes forage and seed yield data from these tests and presents observations of plant type and growth behaviour made at the Dominion Forage Crops Laboratory, Saskatoon, Sask.

LITERATURE REVIEW

The occurrence of reasonably distinct types within the species *Bromus inermis* Leyss. which differ in morphology and adaptation was first pointed out by Zerebina (12, 13, 14). Zerebina made an extensive collection of native and cultivated brome grass strains within different districts of the U.S.S.R. and studied this material together with small collections from Western Europe, the United States, and Canada at the plant-breeding stations of Detskoe Selo (60° N. latitude) and Kammenaya Steppe (51° N. latitude). Two main ecological-geographical groups were recognized: (1) the "meadow" group or northern climatype, and (2) the "steppe" group or southern climatype. Descriptions of these two groups indicate correspondence with northern and southern types subsequently observed within the United States.

Zerebina observed that roots of the steppe group were distributed at a greater depth in the soil than the roots of the meadow group but there was no consistent difference in the extent of horizontal rhizome development

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⁴ Production Reports. Plant Products Division, Production Service, Dominion Department of Agriculture. 1948.

between the two groups. Plants of the steppe group were slightly shorter and more erect than those of the meadow group. In the steppe group the main level of vegetative tillers was one-half that of the reproductive tillers whereas in the meadow group the main level of vegetative tillers approached two-thirds that of the reproductive tillers. Leaves of the steppe group were coarser in texture, shorter, narrower, and more erect than in the meadow group. They were also darker green in colour and bore more waxy bloom than leaves of the meadow group. Short narrow panicles were more commonly found within the steppe group, but no differences in seed characters could be found. Flowering was later and less prolonged in the steppe group. In regard to disease resistance the steppe group was found more susceptible to rust (*Puccinia bromina* Eriksson) and the meadow group more susceptible to brown spot (*Pyrenophora bromi* Died.).

Native collections showed the distribution area of the meadow type in the U.S.S.R. to extend from Murmansk in the north to the Caucasus in the south although south of the Central Chernozem Region this type was generally confined to valleys and more moist habitats. The steppe type accompanied the meadow type in the Central Chernozem Region and predominated in the dry steppe areas of the mid and lower Volga districts, Kazakstan, the northern Caucasus, the Eastern Ukraine and the southern Altai districts of Asia. Collections of cultivated brome grass from America and most of the collections of cultivated brome grass from within the U.S.S.R. were found to be of the northern or meadow type. Representatives of the true steppe type were rarely found in collections of cultivated brome grass but representatives of an intermediate type approaching that of the southern group were frequently found in cultivated collections from the Central Chernozem Regions, the central and lower Volga districts, and Western Siberia.

In America, Newell and Keim (10) studied a considerable number of brome grass strains obtained from northern and southern seed sources at Lincoln, Nebraska (40° N. latitude) and found strains from southern areas to be much higher in hay production than strains from northern areas. In the first year of production southern strains also yielded more seed but in the second year of production this situation was reversed with northern strains yielding more seed. Southern strains also had more vigorous seedlings which were better able to withstand heat and drought conditions. The more rapid growth of southern strains in the spring was considered an indication that these strains were earlier than northern strains. Additional evidence of the superior adaptation and forage productivity of the southern type in the States of Kansas, Iowa, Ohio, and Missouri has been given by Anderson (1, 2), Wilsie *et al.* (11), Lambert (9), and Brown (4). The varieties Achenbach, Fischer, Lincoln, and Elsberry are certified varieties of the southern type which have been developed within these states mainly from old established fields. In Michigan, Churchill (5) found the advantages of southern strains of brome grass to be less evident. Although these strains were more aggressive and higher in forage yield the lower protein content of the southern strains resulted in a lower total production of protein per acre than was obtained from northern strains.

Newell and Keim (10), Anderson (1), and Hansen (8) have reviewed the history of the introduction of brome grass to the United States. Southern strains were shown to arise from introductions from France and Hungary around 1880, while northern strains came from importations from Russia during the period 1896-1898. Introductions from Russia were on a large scale and extensively distributed with the result that brome grass of the northern type soon predominated in the American seed trade. In Canada, Fletcher (7) reported the introduction of brome grass from Germany about 1888 and the distribution of seed samples throughout all of Canada. It was the one grass above all others introduced to find uniformly good acclaim in Western Canada.

MATERIALS AND METHODS

Seed of the southern strains included in these tests was provided by the United States Department of Agriculture and the Agricultural Experiment Stations of states developing these strains. Seed of the northern commercial type was obtained locally at each station conducting a test, while seed of named and numbered strains of the northern type was obtained from the University of Saskatchewan, Saskatoon, Sask., and the Dominion Forage Crops Laboratory, Saskatoon, Sask.

Tests were conducted at the Dominion Experimental Stations at Beaverlodge, Lacombe, and Lethbridge in the Province of Alberta; at Swift Current, Melfort, Saskatoon, and Indian Head in the Province of Saskatchewan; and at Brandon and Morden in the Province of Manitoba. These stations represent a range in latitude from 49° N. latitude at Morden to 55° N. latitude at Beaverlodge. The stations at Lethbridge, Swift Current, and Saskatoon are within the drier brown and dark brown soil zones while the remaining stations are within the more moist black soil zone. The soil types represented by these stations vary from clays to clay loams.

The type of test employed, the varieties included, and the method of establishment varied from station to station. In general, however, randomized block designs were used and four or six replications provided. All tests were sown in the spring as is the general practice in this area and first yields taken in the second year of establishment. At all stations comparisons were made with brome grass growing as the pure species but at Indian Head, Saskatoon, and Brandon comparisons were also made with brome grass growing in combination with alfalfa.

Varieties have been designated as northern or southern in type according to the similarity of plant type and frost reaction to that of northern commercial brome grass or the Achenbach strain of the southern type. In certain cases stations originating strains were consulted as to type. The distinction of strains on the basis of frost reaction was considered quite critical as southern strains showed distinct superiority in the ability to remain green in the fall after the first light frosts had occurred and in the ability of overwintering stands to recommence growth in the spring.

Hay Production

RESULTS

Twelve tests of hay production of northern and southern types were carried out by the nine stations conducting tests. Although the first test was sown in 1938 most of the tests were carried out from 1944-1948. A summary of hay production in these tests is presented in Table 1 along with information on the nature of the tests and the latitude of the testing stations. In recording the results for each test varieties were grouped into the northern and southern types and the average yield of each brome grass strain for the full period of testing was expressed as a percentage of the yield of the northern commercial strain. Statistical analyses of results were not carried out for certain stations and due to the fact that different strains entered the various tests an analysis of combined results was not possible. It should be pointed out that in these tests strains were grown as the single species and that only one cutting per year was obtained except for the irrigated test at Lethbridge where two cuttings were obtained in two of the three years of testing.

No consistent advantage was shown for either the northern or southern type in production of forage although at certain stations the northern type was significantly higher in yield and at others the southern type was significantly higher in yield. As an average of the 11 tests in which both the northern commercial strain and the Achenbach variety were compared the yields of forage were almost equal. At Saskatoon, Lethbridge, Swift Current, Brandon, and Morden southern strains on the average yielded more than northern strains, while at Beaverlodge, Lacombe, and Indian Head northern strains on the average outyielded southern strains. It is difficult to explain the better performance of either type at certain stations in terms of latitudinal or climatic differences. In general the northern type yielded better at more northern stations and the southern type better at more southern stations. The low average yield of northern commercial in pounds of hay produced per acre shown in certain tests at Saskatoon, Swift Current, and Morden is indicative of the dry conditions under which tests were run at these stations. The relatively good performance of the southern type at these stations may be taken as an indication of greater drought resistance in this type. On the other hand at Lethbridge under irrigated conditions, where drought was not a factor, southern strains still outyielded northern strains.

At the Swift Current station much better persistence was found for the Achenbach strain than for the northern commercial strain or the Parkland variety. In a test sown in 1938 it was found by 1948 that stands of both northern strains had been thinned with an ingress of other species while stands of the Achenbach strain were complete in all replicates. This observation would also indicate superior adaptation of the southern type to regions of low rainfall and high summer temperatures.

In the irrigated test at Lethbridge where two cuttings were obtained in two of the three years of testing the production of hay of both types was rather similar in all cuttings. These results are in contrast to those of Newell and Keim (10) who found southern strains producing over twice as much as northern strains in early cuttings.

TABLE 1.—HAY YIELDS OF NORTHERN AND SOUTHERN BROME GRASS STRAINS AT NINE STATIONS IN WESTERN CANADA. HAY YIELDS EXPRESSED AS PERCENTAGES OF NORTHERN COMMERCIAL BROME GRASS

Strain	(1) Beaver- lodge	(2) Melfort	(3) Lacombe	(4) Saska- toon	(5) Saska- toon	(6) Saska- toon	(7) Indian Head	(8) Leth- bridge	(9) Swift Current	(10) Swift Current	(11) Brandon	(12) Morden	Average eleven tests
<i>Northern Type</i>													
Commercial	100	100	100	100	100	100	100	100	100	100	100	100	100
Parkland	—	98	100	97	—	96	89	—	96	104	98	117	—
Superior	—	193	—	—	94	100	—	—	—	98	—	—	—
S-23-7	—	101	—	—	—	—	89	—	—	91	—	—	—
S-23-12	—	—	107	—	—	—	95	95	—	—	85	103	—
Western Iowa	—	—	104	—	—	98	—	96	—	—	87	—	—
Iowa—1158	—	—	—	109	84	—	—	—	—	—	—	—	—
Average	100	100	103	102	93	98	93	97	98	98	92	107	—
<i>Southern Type</i>													
Achenbach	—	96	83	113	84	103	83	102	96	121	113	115	101
Lincoln	70	—	90	108	101	104	99	106	—	—	105	111	—
B. in. -9	95	—	85	116	—	119	93	107	—	—	107	121	—
Fischer	96	—	88	—	—	112	92	108	—	—	113	111	—
N.E. Nebraska	96	—	87	—	—	—	87	108	—	—	122	117	—
Iowa synthetic	80	—	—	—	—	—	—	—	—	—	111	—	—
Elisberry	—	—	—	—	98	—	—	—	—	—	—	—	—
Average	87	96	87	112	94	110	91	106	96	121	112	115	—
Level of significance:	—	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	—	—	—	—	—
Strains	—	n.s.	1%	5%	n.s.	5%	n.s.	1%	—	—	—	—	—
Type	—	—	—	—	—	—	—	—	—	—	—	—	—
Yields of northern com- mercial tons per acre	1.72	1.34	1.74	1.15	1.44	1.32	2.04	2.86	1.15	0.43	1.87	0.59	
per cut	2	4	3	4	3	2	3	3	4	3	3	3	
Years of testing	1	4	4	6	4	6	4	4	4	4	4	4	
Replications	—	—	—	—	—	—	—	—	—	—	—	—	
Latitude of testing station degrees	55	53	52	52	52	52	51	50	50	50	50	49	

Within the group of northern strains no strain has shown any superiority over the commercial strain in general use. The Parkland and Superior varieties and strains S-23-7 and S-23-12 which were the result of selection for the reduced creeping habit are slightly lower in yield than the commercial type. The two strains of the northern type coming from Iowa also appeared similar to the northern commercial type in forage production. Within the group of southern strains no strain appeared to excel other strains of the group in forage yield.

Seed Production

Observations of seed production were made in conjunction with tests of hay production at Beaverlodge, Saskatoon, and Morden. In the test at Beaverlodge and the three tests at Saskatoon actual seed yields were obtained while at Morden seed yields were estimated on the basis of seed culm production. Yields of seed for these tests are summarized in Table 2. Strains have been grouped according to type and the production of each strain has been expressed as a percentage of the production of the northern commercial strain.

TABLE 2.—SEED YIELDS OF NORTHERN AND SOUTHERN STRAINS OF BROME GRASS AT THREE STATIONS IN WESTERN CANADA. SEED YIELDS EXPRESSED AS PERCENTAGES OF THE YIELD OF THE NORTHERN COMMERCIAL TYPE

Strain	(1) Beaver- lodge	(2) Saskatoon	(3) Saskatoon	(4) Saskatoon	(5) Morden	Average 4 tests
<i>Northern Type</i>						
Commercial	100	100	100	100	100	100
Parkland	—	29	—	59	100	—
Superior	—	—	95	122	—	—
S-23-12	—	—	—	—	96	—
West Iowa	—	—	—	110	—	—
Iowa-1158	—	74	92	—	—	—
Average	100	68	96	98	99	100
<i>Southern Type</i>						
Achenbach	—	49	57	49	47	51
Lincoln	56	28	41	64	61	44
B. in. —9	81	31	—	71	61	—
Fischer	56	—	—	82	53	—
N.E. Nebraska	91	—	—	—	53	—
Iowa synthetic	78	—	—	—	—	—
Elsberry	—	—	57	—	—	—
Average	72	36	52	66	55	48
L.S.D. strains per cent (P = 0.05)	—	35	24	38	—	—
Yield of northern com- mercial in lb. per acre	605	85	616	109	—	—
Years of testing	2	3	1	1	3	—
Replications	1	6	4	18	4	—

Marked differences were found in the seed production of northern and southern types in all tests. In no test has any strain of the southern type equalled the yield of the northern commercial type nor has any other strain

of the northern type significantly outyielded the northern commercial strain. On the basis of four tests the Achenbach variety yielded 51 per cent of the northern commercial type and the Lincoln strain 44 per cent of the northern commercial type. These results are in marked contrast to the results of Newell and Keim (10) who found that the seed yields of the southern type were significantly superior to the yields of the northern type at least in the first year of production. It was observed in tests at Saskatoon that seed yields from all strains were greatest in the first year of harvesting and the superiority of the northern types was most apparent in that year. Seed yields of the strain from western Iowa and strain 1158 from Iowa were comparatively high, thus indicating a preservation of the characters of the northern strains even though these strains were maintained for some time in southern areas. The Parkland variety generally recognized as a low seed producing strain showed very low seed production in two tests at Saskatoon.

Mixtures with Alfalfa

In view of the recognized advantages of growing northern brome grass in combination with alfalfa it was desirable to test strains of the southern type in mixtures with alfalfa also. The dense sod forming characteristic of the southern strains suggested that these strains might prove unusually competitive in combination with alfalfa thereby reducing the proportion of alfalfa in the mixture and the consequent advantages arising from the legume. Tests at Indian Head and Brandon were made of northern and southern strains in mixture with Grimm alfalfa. In these tests no advantage was shown for northern or southern strains in the mixture as far as total yields of the mixture were concerned. Unfortunately no separation analyses were carried out to show the proportion of legume in the mixture but general observation indicated no strain as being so aggressive as to eliminate alfalfa from the mixture. A test of mixtures was sown at Saskatoon in 1946, but the proportion of alfalfa in the mixture has remained small and the test has been essentially one of the grass alone. Separation analyses were made in this case and these showed all strains of brome grass of both southern and northern types to have similar amounts of alfalfa in the mixture except for the Parkland strain where the amount of alfalfa was significantly greater than for any other strain.

Morphological and Physiological Differences

Differences in leaf, stem, and panicle characteristics of northern and southern types as noted by Zerebina (12) were found to correspond reasonably well with differences between northern and southern types observed in these studies. Figures 1 and 2 show typical plants of the two types under conditions at Saskatoon. The most marked difference between plants of the two types was in the nature of the leaf. Leaves of the southern type were borne at a lower level on culms and were wider, coarser, and more glaucous than leaves of the northern type. Panicles of the southern type were more contracted and at maturity generally showed less anthocyanin development. Seed produced from northern and southern types in variety tests at Saskatoon was checked for bushel weight and weight per thousand seed and no differences found. Seed of the southern strains appeared more



FIGURE 2. Typical plant of the southern type.



FIGURE 1. Typical plant of the northern type.

chaffy, however, due to the more flaring lemma margins of seeds. A critical comparison of rhizome development in single plants in the second year of growth did not indicate a significant difference between northern commercial and the Achenbach variety in the extent of rhizome development. However, southern strains were observed to form a denser turf which was more difficult to plow up after the grass had been down several years. No difficulty has been experienced at Saskatoon in the eradication of either type.

The southern type at Saskatoon flowered two to four days later than the northern type and consequently may be considered slightly later at Canadian latitudes. Later flowering for the southern type was also observed by Zerebina (12) at somewhat comparable latitudes. Newell and Keim (10) and Lambert (9) considered northern strains later than southern strains on the basis of retarded development of the northern strains in the spring of the year. Under greenhouse conditions, Evans and Wilsie (6) and Atwood (3) found the northern type to be earlier than the southern type.

Two diseases causing leaf deterioration of brome grass at Saskatoon, particularly in years of high rainfall, are brown-spot caused by *Pyrenophora bromi* Died., and leaf-blotch caused by *Selenophoma bromigena* (Sacc.) Sprague and A. G. Johnson. In 1945, moderately severe infection of leaf-blotch in single plant nurseries and solid-seeded plots allowed observations of strain susceptibility. A strain test of northern and southern types sown the previous year was examined and strains scored for resistance to leaf-spot damage. Table 3 presents the average rating of strains included in this trial.

All strains of the southern type were significantly higher in resistance to leaf-blotch than the northern commercial strain. No significant difference could be found between strains within either type in resistance to this disease. Rust damage observed to be severe on brome grass of the southern type in the U.S.S.R. (12), was not observed on either type of brome grass at Saskatoon.

TABLE 3.—RESISTANCE OF BROME GRASS STRAINS TO DAMAGE BY LEAF-BLOTCH (*Selenophoma bromigena*), SASKATOON, 1945. STRAINS RATED FROM 1 (SEVERE DAMAGE) TO 5 (NO DAMAGE)

Strain	Damage score
<i>Northern Type</i>	
Commercial	3.0
Parkland	3.2
Iowa-1158	3.0
Average	3.1
<i>Southern Type</i>	
Achenbach	4.2
Lincoln	4.2
B. in. -9	3.8
Average	4.1
L.S.D. strains ($P = 0.05$)	0.8



FIGURE 3. Test of northern and southern strains of brome grass at Saskatoon in the fall of the first year of growth. Note uniformly good establishment of all strains. *Left plot (47) northern commercial; right plot (46) Neb. 44.*

Newell and Keim (10), Anderson (1), and Lambert (9) have indicated that in fall seedings under southern conditions much greater seedling vigour is shown by southern strains. In spring seedings at Saskatoon no difference has been noted in field trials in the vigour of seedlings or uniformity of stands between southern and northern types. In 1948 a six-replicate plot test of southern and northern strains was sown on May 18 and harvested on August 20 to obtain information of the rapidity of establishment. Varieties of the northern type in this test were northern commercial, Parkland, and Martin. Varieties of southern type were Achenbach, Lincoln, Neb. 36, and Neb. 44. Yields of over one ton per acre were obtained but no significant difference was found between strains. Figure 3 gives a general view of this test prior to cutting for hay and indicates the overall good establishment of strains.

Self- and Cross-Fertility

During 1945 and 1946 a total of 95 plants of the northern type and 59 plants of the southern type were observed for self- and open-fertility at Saskatoon. Determinations of self-fertility were made on the basis of seed-setting within three parchment bags while determinations of open-fertility were made on the basis of seed-setting of duplicate samples of 10 open-pollinated panicles. Table 4 shows the distribution of plants according to self- and open-fertility ratings.

It is apparent from Table 4 that there is little difference between northern and southern types in self-fertility or open-fertility as shown either by the average set of seed or the distribution of plants for level of

TABLE 4.—SELF- AND OPEN-FERTILITY RATINGS OF NORTHERN AND SOUTHERN TYPES OF BROME GRASS. PLANTS RATED ACCORDING TO THE NUMBER OF SEEDS FORMED PER PANICLE. SASKATOON, 1945-1946

Type	Percentage of plants with fertility ratings of:									Average fertility
	0.0	0.1-1.0	1.1-10.0	10.1-20.0	20.1-40.0	40.1-80.0	80.1-120.0	120.1-200.0	No. of plants	
<i>Self-fertility</i>										
Northern	3	13	48	18	11	6	1	—	95	10.9
Southern	10	13	46	20	7	2	—	2	59	9.9
<i>Open-fertility</i>										
Northern	—	—	1	2	11	39	32	15	95	77.2
Southern	2	2	2	2	13	35	24	20	59	79.2

seed setting. It is interesting to note that under open-pollination both types produced about an equal number of seeds per panicle. Since the southern type has been shown to be lower in seed production than the northern type, it would appear that this was due to the production of fewer seed culms rather than due to the presence of smaller panicles or sterility in the southern type. The failure to obtain any difference in self-fertility between the two types is in keeping with the results of Zerebina (12), and Lambert (9).

In 1948 the ability of the two types to intercross was studied at Saskatoon by pollinating two plants of the southern type and one of the northern type with pollen from four plants of the southern type and six plants of the northern type. The method of crossing consisted of placing detached panicles of the ten pollen parents within separate pollination bags applied previously to the seed parents. This transference was made just after first flowering had taken place and transferred panicles were kept alive in the new environment by placing the bases of stems in water. Not all combinations were completed due to a failure to obtain complete correspondence in flowering and due to the loss of several combinations from wind damage. Table 5 presents data showing the success of seed setting on the crosses that were harvested.

TABLE 5.—INTERFERTILITY OF NORTHERN AND SOUTHERN TYPES OF BROME GRASS AS SHOWN BY CONTROLLED POLLINATIONS OF SELECTED PLANTS, SASKATOON, 1948

Type of cross	Number of crosses	Average seed-setting in terms of seeds per panicle		
		Controlled crosses	Female parents on	
			Selfing	Open-pollination
Northern × northern	7	62	5	75
Northern × southern	3	38	5	75
Southern × southern	6	69	18	105
Southern × northern	13	66	18	105

While plants used as female parents in these crosses were not completely self-sterile the increase in seed setting following cross-pollination is indicative of a high degree of crossing. Since the success of seed setting following crossing of different types approaches that from crossing similar types it would appear that the two types of brome grass are cross-compatible. This fact is of significance to the plant breeder wishing to combine the favourable characteristics of the two types.

DISCUSSION

Since southern strains of brome grass have been found inferior in seed yields in these studies it appears undesirable to distribute or license the sale of these varieties in Western Canada at the present time. Unless much stronger discrimination against Canadian brome grass seed develops within the United States than at present exists growers in Western Canada are not likely to show interest in the southern type. If southern strains were introduced into the present seed areas with a view to seed production the preservation of strain identity would likely be difficult due to the wide distribution and good adaptation of the northern type. However, the advent of varieties of the southern type with good seed yields might warrant production of these varieties outside the present seed districts. The drier brown soil zone where the southern type has shown some advantage in yields of forage and where the northern type is not widely grown at present might be considered as an area for the production of southern strains.

The greater disease resistance of the southern type is a character making the southern type of interest in the brome grass improvement programme. At the Dominion Experimental Farm at Brandon and the Dominion Forage Crops Laboratory at Saskatoon selection is being carried on within both the northern and southern types for plants with high forage and seed yields and for resistance to leaf-spot diseases. In view of the fact that most of the brome grass seed produced in Western Canada is being sold on markets in the United States and that bred strains from Canada have performed particularly poorly in the United States, it is imperative that new varieties be acceptable in the United States before being distributed in Canada.

Named and numbered strains of the northern type have shown no superiority in forage or seed production over the northern commercial type and this fact may be taken as a reflection on the methods of producing these strains. The Superior variety was developed by the mass selection of a small number of plants of the northern type and differs from the commercial type in being slightly less strongly creeping. The Parkland variety and strains S-23-7 and S-23-12 were developed by repeated selection within inbred lines and are definitely less strongly creeping than the original commercial type. Had tests of combining ability been applied in the production of these strains higher yields of seed and forage might have been obtained. It should be pointed out that these four strains form a negligible part of the Canadian seed trade at the present time. The Parkland variety which showed promise as a less strongly creeping variety is no longer recommended in Saskatchewan in view of its low seed yields. No

investigations have been made of the occurrence of regional strains in Western Canada but general observations of farm fields and experimental plots have indicated no deviation from the general northern type.

The history of the introduction of brome grass to America as presented by Newell and Keim (10), Anderson (1), and Hansen (8) is substantiated by these studies in that two reasonably distinct forms were indicated and correspondence found with similar types in Europe. The southern type which is adapted to the arid steppe regions of the U.S.S.R. was found to be well adapted to the drier zones in Western Canada. The northern type occurring more commonly on alluvial plains and moist meadows in the U.S.S.R. was found to yield somewhat better than the southern type at certain stations within the more moist park belt in Western Canada. Newell and Keim, and Anderson have suggested France and Hungary as sources of the southern strains in America. The occurrence of southern types within the U.S.S.R. as pointed out by Zerebina (12) would suggest that southern types or intermediate forms may have been introduced to America from Russia along with the northern type.

The designation of the two types of brome grass as "southern" and "northern" would infer more marked differences in adaptation and photo-periodic response of the two types than shown in these tests. Southern strains in these tests were fully as hardy as the northern strains and on the average as productive of hay. The marked difference in seed production of the two types might be considered an indication of a differential photo-periodic response but the dates of flowering for the two types differed little. In view of the lower seed yields of southern strains in the second year of production under Nebraska conditions (10) it is probable that southern strains are characteristically low in seed production. A similar response of the two types in Western Canada might be expected in view of the fact that the northern limits of brome grass distribution in Europe lie considerably north of the northern limits of cultivation in America. At latitudes comparable to those of the Canadian stations both northern and southern types occur naturally and are cultivated in the U.S.S.R. Zerebina (12, 13, 14) has indicated that in the Central Chernozem Region of the U.S.S.R. the occurrence of either type is governed by ecological adaptation, particularly adaptation to moisture conditions. From these studies it would appear that a somewhat similar distinction with regard to drought tolerance might be made in Western Canada.

SUMMARY

1. Forage production of southern strains of brome grass at nine stations in Western Canada was found to be similar to that of northern commercial brome grass.

2. Seed production of southern strains at three stations in Western Canada was found to be inferior to that of northern commercial brome grass. The yield of seed of the southern strains Achenbach and Lincoln was about half that of northern commercial brome grass.

3. Northern and southern strains reacted similarly in mixtures with alfalfa as far as total yields were concerned. Tests of mixtures have not been continued for a sufficient period of years to conclude as to the aggressiveness of strains in mixtures.

4. Plants of the southern type showed minor variations in plant type from the northern type particularly with respect to the nature of the leaf and panicle shape.

5. Southern strains were two to four days later in flowering than northern strains and showed more resistance to fall and spring frosts. Southern strains possessed superior resistance to certain leaf spot diseases.

6. The degree of self-fertility and the distribution of plants for level of self-fertility was similar for the two types. In controlled crosses the two types were found to be interfertile.

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ERRATUM

In the article published in the July issue of *Scientific Agriculture*, Volume 29, pp. 345-350, "Prevention of Early Decay of Cut Potato Sets by Chemical Treatment", by G. B. Sanford, an error occurred in the caption for Figure 1. This should read: "Figure 1—A. . . . *fourth row*, uninfested soil, sets not treated; *bottom row*, infested soil, sets not treated."

SHATTERING, BREAKING AND THRESHABILITY IN BARLEY VARIETIES¹

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INTRODUCTION

Shattering and breaking in barley have always caused important losses to growers in the open plains region of Western Canada. This damage is caused by wind or by wind and rain as the crop nears maturity or after it matures. The straw may be broken over, the spike broken off or the seed lost from the spike. Such damage has no relation to lodging. Formerly these losses were not so important because acreages of barley were small and it was all harvested with binders at or just before maturity. Under such conditions it was only in the occasional field that serious losses occurred. However, for a number of years much larger acreages have been grown and practically all the crop has been harvested with combines. As the crop must be thoroughly mature and dry before this method of harvesting is successful, it has been exposed to damage, often for periods of several weeks, at a time when it is very susceptible. Swathing the crop to avert damage has reduced losses in most cases. However, light crops cannot be swathed satisfactorily as difficulty is experienced in picking up thin swaths. Moreover, losses still occur because large acreages cannot be readily covered in a short period of time.

Observations on shattering and breaking in barley were commenced at Swift Current Experimental Station in 1942. In that year extensive damage occurred to the varietal test plots. It was noted that different types of damage occurred and that varietal differences existed. Since that year notes on the various types of damage that occurred to each variety in test plots have been taken each year. The object was to determine the different types of damage, something of the factors affecting damage and, more particularly, the best sources of resistance that might be used in a breeding program.

Recently the variety Titan has become available for commercial production. It has high resistance to shattering and breaking. Unfortunately, in commercial production this variety was found difficult to thresh, which suggested the possibility that in breeding for resistance to shattering a problem in threshing might arise. Accordingly, attempts were made to evaluate the threshability of varieties and to relate this to their tendency to shatter.

This whole study was necessarily of an exploratory nature, but the information obtained should be of value in planning more precise experiments.

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TYPES OF DAMAGE

The terminology used to describe damage to cereal crops caused by weather conditions from the time the crop nears maturity until it is harvested does not appear to be well established. Accordingly, the terms used in this paper are defined below. They appear to be reasonably suitable when applied to barley and may or may not have application to other crops.

Shattering—refers to loss of seed from the spike. In these experiments all such losses were classified as shattering loss. It has recently been observed, however, that such losses occur two ways. In the first, individual seeds become detached from the rachis and fall to the ground. This damage is characteristic of the varieties Prospect and Bay. In future experiments it is proposed to reserve the term "shattering" for this type of damage. In the second type the rachis breaks and part of the spike is lost. This is characteristic of the variety Warrior. It is proposed, in future experiments, to refer to this as "rachis breaking".

Damage to the straw has been classified into three types.

Neck breaking—refers to a break in the upper internode, almost always near the spike, with the spike usually falling to the ground or, occasionally, hanging down the side of the stem.

Stem breaking—refers to definite breaks in the stem at any point below the upper internode. Such damage has been observed only following prolonged winds of high velocity.

Buckling—refers to a bending or partial breaking, usually at a number of points in the stem. In extreme cases the spike may touch the ground but does not become detached from the stem. The term "buckling" was used because it describes a condition similar to that occurring in wheat and described by Fellows (3) as "buckling". Various authors have referred to "crinkling" in small grains, and in some cases they may have had in mind the condition described above.

METHODS

The data obtained were taken on the border rows of the regular varietal tests. The varieties were grown in three rod-row plots replicated six times. Data from the dryland tests were obtained in each of the years 1943 to 1947. In all of those years the crop suffered considerably from drought. During the years 1945 to 1947, data were also obtained from the varietal tests grown under irrigation. Compared with the dryland tests those irrigated were under different soil and moisture conditions but were subjected to approximately the same weather conditions at all times.

On the average, the data were taken about two weeks after the latest variety in the test had matured. Following the harvesting of the centre row the borders were kept under observation, and when a good differential was evident the notes were taken. In some years this occurred at maturity and in others almost a month might have elapsed. Notes on all varieties were taken on the same day. Hence, early-maturing varieties were exposed to damage for a longer period than late ones. While this was undoubtedly a source of error in evaluating varietal reaction, it appeared to be small in

relation to the differences between varieties. In fact, the most highly resistant varieties were among the earliest in the tests. As the notes were not taken until a differential occurred, the values obtained are in excess of normal field losses by substantial amounts. Field losses as great as any reported do occur, however, when the crop is not harvested promptly or when unfavourable weather intervenes.

In evaluating shattering, the percentage of seeds lost from the spike was estimated. No attempt was made to differentiate between losses due to the seeds becoming detached from the rachis and losses due to rachis breaking. It was not realized until recently that the type as well as the amount of shattering might be a varietal characteristic. Neck breaking and stem breaking were evaluated either by making counts or by estimating the percentage broken. When estimating shattering or breaking, the mean of two independent estimates was used in practically all cases. No attempt was made to assign numerical values to buckling damage that occurred.

In evaluating ease of threshing, the samples from the centre rows of the plots were air-dried and then threshed in a Kemp thresher. This machine has been described by Kemp (6). In 1946, lots of one hundred seeds each were taken from each of three replicates. The percentage of threshed and unthreshed seed was noted. A seed was considered unthreshed if more than 3 mm. of awn remained or if any of the rachis was adhering. Since there was no evidence of any significant variation due to replicates in this test, lots of one hundred seeds each were taken from a bulk sample from the six replicates in 1947. This test was conducted for only the two years and only on the dryland samples.

In analysing the data Goulden (4) has been used as a guide. As the values obtained were percentage figures and as zero readings were sometimes obtained, the value "1" was added to all readings and these were then converted to $\sin^2\theta$. To establish the significance of varietal differences the results of each test were analysed by variance. It was not possible to group the tests because the varieties differed from year to year. The inter-relationships of the various characters were studied by means of covariance except for the data on threshability. As these data were not taken on individual plots the covariance technique was not applicable. In this case the correlation coefficient was used.

SHATTERING

Some of the statistics calculated from the data on shattering are presented in Table 1. In all tests highly significant varietal differences were established. In most tests the magnitude of the differences was substantial, indicating that they are of importance in evaluating varieties for commercial production.

Eight varieties were grown on both irrigated and dryland for the three years 1945-47. The average percentage shattering for these varieties in both locations is presented in Table 2. Shattering on irrigated land was much greater than on dryland. Only one of the varieties shattered appreciably on dryland whereas only one showed high resistance to shattering on irrigated land.

A summary of results obtained with a number of named varieties grown on dryland is presented in Table 3 and with some grown on irrigated land in Table 4. The variety Glacier was outstanding in that it was resistant in all tests. Such varieties as Titan and Trebi were highly resistant in the dryland tests but showed some susceptibility in the irrigated tests. Warrior was resistant in some tests on dryland and susceptible in others. It was not tested under irrigation. Montcalm and O.A.C. 21 were susceptible in all tests in which they were included. Prospect was also highly susceptible to shattering.

TABLE 1.—MEAN PER CENT SHATTERING AND RANGE OF SHATTERING AMONG BARLEY VARIETIES GROWN ON DRYLAND, 1943-1947, AND ON IRRIGATED LAND, 1945-1947, TOGETHER WITH CONVERTED DATA ($n + 1 = \sin^2\theta$) AND STANDARD ERRORS AND F VALUES CALCULATED FROM THE CONVERTED DATA

Year	No. of vars.	Mean		S.E. in per cent	Min. sig. diff.	Range (actual)		Range (converted)		F ¹
		Actual	Converted			Low	High	Low	High	
<i>Grown on dryland</i>										
1943	17	7.0	14.1	7.38	2.9	1.5	15.0	7.1	23.2	30.47
1944	26	9.3	16.8	13.08	6.2	0.0	34.1	5.7	36.2	11.85
1945	49	7.1	14.4	13.96	5.6	0.0	42.5	5.7	41.2	13.38
1946	42	12.5	18.3	17.50	8.9	0.0	49.2	5.7	45.0	12.82
1947	36	19.1	22.7	11.41	7.2	0.0	77.5	5.7	62.8	40.15
<i>Grown on irrigated land</i>										
1945	36	18.3	21.6	14.97	9.1	0.0	67.5	5.7	56.6	26.08
1946	28	8.9	15.5	13.40	5.8	0.0	50.8	5.7	46.0	25.14
1947	30	13.0	18.7	14.76	7.7	0.0	50.8	5.7	46.1	17.22

¹ All F values exceed the 1 per cent point.

Because different varieties were included each year it is not possible to use the data in Table 1 for evaluating the effect of years. The results in Tables 3 and 4 show clearly, however, that the amount of shattering varies from year to year on both irrigated and dryland. This is in line with general farm experience. Two main factors appear to be involved. The first of these has to do with the condition of the crop. If the crop matures normally so that large plump kernels are produced, it seems to be particularly susceptible to shattering. On the other hand, if drought intervenes and the crop ripens prematurely with shrunken kernels, it is highly resistant to shattering. The second factor is weather conditions, particularly wind, at the time the crop is ripe. The interactions of these two factors largely determine the amount of shattering that occurs. Thus, in the dryland test in 1943 the crop was subjected to severe drought and little wind was experienced during the harvesting period, with the result that very little shattering was experienced. On the other hand, in 1947, the irrigated test was subjected to severe winds and, as drought was not a factor, shattering was very severe.

TABLE 2.—AVERAGE PER CENT SHATTERING, 1945-1947, FOR VARIETIES GROWN ON IRRIGATED AND DRY LAND

Variety	Per cent shattering, 1945-1947	
	Irrigated	Dry
Glacier	1.7	0.1
Hybrid 36-1991	5.3	0.3
Velvon	15.0	1.0
Vantage	15.7	1.3
Trebi	16.1	1.6
Titan	17.1	0.7
Plush	20.5	4.2
Newal	34.1	19.2
Average	15.7	3.6

TABLE 3.—PER CENT SHATTERING IN BARLEY VARIETIES GROWN ON DRYLAND AT SWIFT CURRENT, 1943-1947

Variety	C.A.N.	Per cent shattering						
		1943	1944	1945	1946	1947	Av. 5 yr.	Av. 3 yr.
Glacier	1149	0.7	0.0	0.2	0.0	0.0	0.2	0.1
Titan	1164	1.5	2.3	0.2	0.8	1.2	1.2	0.7
Trebi	1115	2.2	2.3	1.5	1.7	1.7	1.9	1.6
Plush	1117	2.7	3.1	1.7	4.2	6.7	3.7	4.2
Rex	1113	1.8	9.5	3.2	4.2	14.2	6.6	7.2
Newal	1089	8.2	7.0	8.5	10.0	39.2	14.6	19.2
Warrior	1144	2.3	9.5	8.5	10.8	50.8	16.4	23.4
Montcalm	1135	12.0	12.8	15.3	20.0	37.5	19.5	24.3
Prospect	1140	5.0	30.0	15.0	45.0	29.2	23.9	29.7
Atlas	702	—	—	1.3	0.8	0.0	—	0.7
Compana	1154	—	—	1.8	0.0	0.8	—	0.9
Vance Smyrna	—	—	—	1.3	0.8	0.8	—	1.0
Vantage	1162	—	—	0.6	1.7	1.6	—	1.3
Tregal	1150	—	—	4.3	7.5	12.5	—	8.1
O.A.C. 21	1086	—	—	17.2	44.2	19.2	—	26.9

TABLE 4.—PER CENT SHATTERING IN BARLEY VARIETIES GROWN ON IRRIGATED LAND AT SWIFT CURRENT, 1945-1947

Variety	C.A.N.	Per cent shattering			
		1945	1946	1947	Average
Frontier	110	0.3	0.8	1.7	0.9
Glacier	1149	2.5	0.0	2.5	1.7
Lico	1152	4.7	11.7	20.0	12.1
Sanalta	1088	26.7	5.0	8.3	13.3
Velvon No. 11	1151	7.5	15.8	21.7	15.0
Vantage	1162	10.5	13.3	23.3	15.7
Trebi	1115	9.2	10.0	29.2	16.1
Titan	1164	13.0	15.8	22.5	17.1
Plush	1117	13.0	16.7	31.7	20.5
Newal	1089	28.0	24.2	50.0	34.1

NECK BREAKING

Some of the statistics calculated from the data on neck breaking are presented in Table 5. As was the case in the shattering test, highly significant varietal differences were established in all tests and these differences were of sufficient magnitude to be of economic importance.

Mean values for the percentage neck breaking for the eight varieties common to the irrigated land and dryland tests for the three years are presented in Table 6. Unfortunately, all these varieties, except Newal, are quite resistant to neck breaking so that a comparison of irrigated and dryland results is difficult to make. The evidence available suggests that, in contrast to shattering, breaking is more likely to occur under dryland conditions.

A summary of results obtained with a number of named varieties grown on dryland is presented in Table 7 and with some grown on irrigated land in Table 8. Glacier was highly resistant in all tests. The Manchurian types as a group appear to be very susceptible. No variety of this type has been found to have a high degree of resistance. Differences within the group do occur, however; O.A.C. 21 appears to be the most susceptible whereas Montcalm is somewhat more resistant. Newal and Tregal are two other varieties that show considerable susceptibility to neck breaking.

TABLE 5.—MEAN PER CENT NECK BREAKING AND RANGE OF NECK BREAKING AMONG BARLEY VARIETIES GROWN ON DRYLAND, 1943-1947, AND ON IRRIGATED LAND, 1945-1947, TOGETHER WITH CONVERTED DATA ($n + 1 = \sin^2\theta$) AND STANDARD ERRORS AND F VALUES CALCULATED FROM THE CONVERTED DATA

Year	No. of vars.	Mean		S.E. in per cent	Min. sig. diff.	Range (actual)		Range (converted)		F ¹
		Actual	Converted			Low	High	Low	High	
<i>Grown on dryland</i>										
1943	17	33.5	34.7	9.21	9.21	7.8	81.7	15.6	65.9	36.81
1944	26	21.1	26.4	14.40	10.64	2.5	43.0	10.2	41.1	5.94
1945	49	13.7	20.7	11.72	6.77	0.0	46.6	5.7	43.4	12.71
1946	42	7.3	14.7	16.70	6.82	0.0	27.5	5.7	32.3	8.42
1947	36	20.9	24.8	14.65	10.16	0.0	74.2	5.7	60.6	16.82
<i>Grown on irrigated land</i>										
1945	36	5.8	13.5	15.70	5.94	0.0	15.0	5.7	23.1	6.41
1946	28	1.9	8.7	20.28	4.93	0.0	6.7	5.7	15.2	2.31
1947	30	4.6	11.6	23.75	7.70	0.0	33.3	5.7	33.9	3.69

¹ All F values exceed the 1 per cent point.

In considering the effect of environment on neck breaking it appears that premature ripening of the crop predisposes it to damage. This is in direct contrast to shattering. If the crop is in this condition there is usually sufficient wind to cause substantial damage in susceptible varieties. Under favourable growing conditions, on the other hand, relatively little damage may be experienced on susceptible varieties even when exposed to severe winds. An example of this occurred in the 1947 irrigated test when Newal was damaged only 5 per cent even though it was severely battered with strong winds.

TABLE 6.—AVERAGE PER CENT NECK BREAKING, 1945-1947,
FOR VARIETIES GROWN ON IRRIGATED AND DRY LAND

Variety	Per cent neck breaking, 1945-1947	
	Irrigated	Dry
Glacier	1.2	0.7
Hybrid 36-1991	2.8	2.1
Trebi	8.3	3.9
Titan	3.9	5.4
Vantage	2.1	5.5
Velvon	4.2	6.1
Plush	5.3	9.6
Newal	7.5	16.4
Average	4.4	6.2

TABLE 7.—PER CENT NECK BREAKING IN BARLEY VARIETIES GROWN ON
DRYLAND AT SWIFT CURRENT, 1943-1947

Variety	Per cent neck breaking						
	1943	1944	1945	1946	1947	Av. 5 yr.	Av. 3 yr.
Glacier	7.0	3.5	1.3	0 0	0.8	2.5	0.7
Warrior	9.3	6.8	1.0	4 2	12.5	6.8	5.9
Titan	11.2	11.3	7.8	2.5	5.8	7.7	5.4
Trebi	16.2	16.8	7.5	1.7	2.5	8.9	3.9
Prospect	12.5	14.5	10.8	2.5	8.3	9.7	7.2
Rex	10.2	17.6	13.3	5.0	5.8	10.4	8.0
Plush	17.5	19.5	11.3	4.2	13.3	13.2	9.6
Newal	39.2	41.5	16.6	11.7	20.8	26.0	16.4
Montcalm	50.0	25.7	36.7	6.7	35.0	30.8	26.1
Atlas	—	—	0.0	0.8	2 5	—	1.1
Vance Smyrna	—	—	5.8	0.0	1.6	—	2.5
Compana	—	—	9.1	0.8	1.7	—	3.9
Vantage	—	—	6.6	3.3	6.6	—	5.5
Tregal	—	—	12.5	6.7	38.3	—	19.2
O.A.C. 21	—	—	46.6	21.7	74.2	—	47.5

TABLE 8.—PER CENT NECK BREAKING IN BARLEY VARIETIES GROWN ON
IRRIGATED LAND AT SWIFT CURRENT, 1945-1947

Variety	Per cent neck breaking			
	1945	1946	1947	Average
Glacier	0.3	0.8	2.5	1 2
Vantage	2.8	1.7	1.7	2.1
Lico	3.3	0.0	4.2	2.5
Titan	8.3	0.0	3.3	3.9
Frontier	9.7	2.5	0.0	4.1
Velvon No. 11	6.7	1.7	4.2	4.2
Plush	7.5	2.5	5.8	5.3
Newal	10.8	6.7	5.0	7.5
Trebi	14.2	3.3	7.5	8.3
Sanalta	14.2	15.0	7.5	12.2

STEM BREAKING

Data on stem breaking were secured on one test only. This was the 1947 test grown on dryland. Just as the majority of the varieties neared maturity they were subjected to a prolonged wind of high velocity. Data on the duration, direction and velocity by hour of this storm are presented in Table 10. A summary of the results obtained with some of the varieties in this test is presented in Table 9. Among the most resistant varieties were Bay, Glacier and Rex. The Manchurian types, particularly O.A.C. 21, were highly susceptible.

TABLE 9.—MEAN PER CENT STEM BREAKING AMONG BARLEY VARIETIES GROWN ON DRYLAND, 1947, TOGETHER WITH CONVERTED MEANS ($n + 1 = \sin^2\theta$)

Variety	Per cent stem breaking	
	Actual	Converted ¹
Rex	2.5	10.0
Prospect	4.2	11.5
Glacier	5.8	14.8
Trebi	8.3	16.1
Plush	13.3	19.6
Warrior	15.0	22.6
Newal	16.7	23.2
Titan	16.7	23.2
Vantage	21.6	27.4
Tregal	27.5	30.0
Compana	40.0	38.8
Vance Smyrna	46.6	43.3
O.A.C. 21	54.2	48.0
Atlas	63.3	53.8

¹ The minimum significant difference between converted variety means is 11.6.

BUCKLING

Among the common, named varieties of barley this type of damage has been observed only in Compana. A few types from the U.S.D.A. world's collection of barleys have also exhibited buckling. All varieties showing buckling have been short-strawed, two-rowed types. The condition became evident only after the varieties were well matured and became progressively worse with time. It does not appear to be directly due to weather conditions but rather to a deterioration and eventual collapse of culm tissue. As the phenomenon does not occur with equal intensity in all tests, it can be presumed that it is modified by environmental conditions. It appears to present no great hazard to commercial production if the crop can be harvested as soon as it is well matured, but heavy losses might occur if this were not possible.

THRESHABILITY

A summary of some of the threshing data is presented in Table 11. Analysis of the data from three replicates of the 1946 test on dryland showed no significant variation due to replicates. Accordingly, in 1947,

TABLE 10.—TOTAL MILES OF WIND PER HOUR PASSING A GIVEN POINT AT SWIFT CURRENT ON JULY 28 AND 29, 1947

(Data kindly provided by the Swift Current branch of the Dominion Meteorological Service)

Date	Time	Direction	Miles per hour
July 28	4 p.m.	S.W.	23
	5 p.m.	S.W.	26
	6 p.m.	W.	36
	7 p.m.	W.	40
	8 p.m.	W.	34
	9 p.m.	W.	28
	10 p.m.	W.	32
	11 p.m.	W.	30
	12 p.m.	W.	26
July 29	1 a.m.	W.	30
	2 a.m.	W.	29
	3 a.m.	W.	21
	4 a.m.	W.	18
	5 a.m.	S.W.	16
	6 a.m.	S.W.	18
	7 a.m.	W.	35
	8 a.m.	W.	35
	9 a.m.	W.	42
	10 a.m.	W.	56
	11 a.m.	W.	40
	12 a.m.	W.	38
	1 p.m.	W.	44
	2 p.m.	N.W.	44
	3 p.m.	W.	45
	4 p.m.	N.W.	36
	5 p.m.	W.	39
	6 p.m.	N.W.	37
	7 p.m.	N.W.	33
	8 p.m.	N.W.	37
	9 p.m.	N.W.	26
	10 p.m.	N.W.	25
	11 p.m.	N.W.	20
	12 p.m.	W.	18

notes were taken on a bulk sample from all replicates. A reasonably good differential was obtained in 1946 but the range was not so great in 1947. Threshed material from the experimental thresher may not be comparable to that from commercial machines, but it seems probable that such would be the case. Titan, Velvon, Glacier, Atlas, and Prospect were the most difficult varieties to thresh in these tests.

Pope (7) and Aberg and Wiebe (1) have shown that environment markedly influences the threshability of a variety. This is apparently brought about by its effect on the deposition of ash in the awn. Brittleness of awn has been shown to be closely associated with ash content by Harlan and Pope (5). Aberg *et al.* (2) have also shown a direct relationship between ash content and threshability.

TABLE 11.—MEAN PER CENT OF KERNELS THRESHING FREELY FROM BARLEY VARIETIES GROWN ON DRYLAND, 1946-1947

Variety	Per cent free threshing		
	1946	1947	Average
Atlas	63	78	70
Glacier	69	76	72
Prospect	61	83	72
Velvon	65	84	74
Titan	65	89	77
Plush	71	94	82
Trebi	75	95	85
Tregal	84	90	87
Vantage	87	92	90
Warrior	89	96	92
O.A.C. 21	92	94	93
Rex	91	97	94
Montcalm	94	95	94
Newal	98	94	96
Vance Smyrna	96	97	96
Compana	95	99	97

TABLE 12.—SUMMARY OF ANALYSIS OF COVARIANCE BETWEEN SHATTERING AND NECK BREAKING IN BARLEY VARIETIES

Year	F values			r_1	r_2
	Between varieties	Error before and after adjustment	Between regressions	Between varieties	Within varieties
<i>Grown on dryland</i>					
1943	8.35**	1.48	54.68**	0.930**	0.135
1944	4.35**	5.31*	3.43	0.560**	0.202*
1945	8.38**	0.32	66.02**	0.669**	0.036
1946	6.69**	1.27	44.17**	0.578**	-0.078
1947	21.19**	45.96*	91.00**	0.290	-0.457*
<i>Grown on irrigated land</i>					
1945	5.39**	0.61	12.07**	0.459**	-0.059
1946	2.14**	0.86	4.24**	0.376**	-0.080
1947	3.65**	0.14	0.76	0.111	-0.031

* Exceed the 5 per cent point.

** Exceed the 1 per cent point.

INTERRELATIONSHIPS

Shattering and Neck Breaking

A summary of the covariance analysis of the data on shattering and neck breaking is presented in Table 12. In five of the eight tests, namely the dryland tests in 1943, 1945 and 1946 and the irrigated tests in 1945 and 1946, there were highly significant positive relationships between these variables.

It is difficult to offer a satisfactory biological explanation for the positive and negative within variety associations between the variables that occurred in the dryland tests in 1944 and 1947. It is doubtful if the

r^2 values can be considered true estimates of the relationship between the variables in these two tests. Neither can an explanation be suggested for the lack of association between neck breaking and shattering in the 1947 test grown on irrigated land.

Neck Breaking and Stem Breaking

Data are available from the 1947 test on dryland only. An analysis of these data by covariance showed a between-variety correlation of 0.334 that just reached the point of significance, and a non-significant within variety correlation of 0.182. The data are not sufficiently extensive to draw definite conclusions, but they suggest that neck-breaking and stem-breaking tendencies are not closely associated.

Shattering and Threshability

The coefficients of correlation between percentage shattered and percentage free threshing in the 1946 and 1947 tests on dryland were 0.326 and 0.427, respectively. Both of these values exceed the 5 per cent point.

DISCUSSION

The results of this investigation have shown conclusively that varietal differences exist in the amount of damage from shattering and neck breaking that occurs under natural conditions. Furthermore, the magnitude of these differences is sufficiently great to be of considerable significance in evaluating varieties for commercial production. In the production of new varieties the desirability of incorporating resistance to shattering and breaking is evident. While fewer data are available on stem breaking and threshability, here again it would appear that important varietal differences exist and that resistance to stem breaking and ease of threshing are characters that should be incorporated into new hybrids.

The association between shattering and neck breaking will be of some assistance in a breeding program. Its degree of usefulness will probably depend upon the varieties used as parents. In any case the association is unlikely to be sufficiently close to warrant selection on the basis of one character only, except possibly in early-generation material.

On the other hand, the association between shattering and threshability* is likely to prove troublesome. The data obtained suggest that the association is not close, but these data are meagre and may not indicate the true extent of the association. It seems probable that varieties can be produced which have resistance to shattering and which thresh satisfactorily, but such plants may be difficult to locate in segregating populations.

The pronounced effect of environment on all characters under discussion will need consideration in the breeding program. It may not be possible to secure a differential for all characters in any one test. The results suggest that differentials for neck breaking and threshability can best be secured under drought conditions while shattering is more pronounced when the crop is irrigated. The influence of different stations even within a natural region, such as the open plains area of Western Canada, has not been investigated but it seems probable that differences would exist. If this is so, then advanced hybrids, at least, should be tested at more than one station.

Undoubtedly improvements can be made in producing differential environments. By choosing locations fully exposed to prevailing winds both neck breaking and shattering would be increased, while the application of water by sprinklers when the crop is mature might increase shattering considerably. There is also the possibility of using mechanical tests and chemical determinations. Apparatus to test breaking strength and to produce shattering mechanically could probably be produced, while the possible relationships between shattering and threshability with ash content of the awns should be investigated. The greatest difficulty with all such tests is likely to be the time required to handle any large bulk of material. If they are developed they will likely be most useful for critical studies of factors affecting the characters involved and for detailed analyses of possible parental material. Field tests will probably remain the most efficient way of eliminating undesirable material from hybrid populations.

SUMMARY

Damage to barley varieties at and following maturity by wind and rain was studied. Shattering and neck breaking were noted in a series of varieties for five years when grown on dryland and for three years on irrigated land. Data on stem breaking were obtained in one test. Buckling was noted on certain varieties. In addition, the threshability of the varieties in two tests was recorded.

Highly significant varietal differences were established in all tests for all of the characters studied. These differences were large enough to be of economic significance. The variety Glacier was highly resistant to all types of damage, while the Manchurian types tended to be highly susceptible.

In most tests shattering and neck breaking were positively associated. Neck breaking and stem breaking, and shattering and threshability were also positively associated.

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DEVELOPMENT OF A FORMULA FOR ESTIMATING SURFACE RUN-OFF¹

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INTRODUCTION

The anticipated rate and volume of surface run-off are essential factors in the design of any water control or storage structure that handles surface water.

A large number of small water conservation and irrigation projects have been built in the three prairie provinces, particularly since the inception of the Prairie Farm Rehabilitation Act in 1935. This type of work will continue in the future, and in addition there is a growing demand for run-off data in relation to water erosion control practices.

With regard to rate of run-off, the designer is primarily interested in the rate of flood flow that may be anticipated to be equalled or exceeded once, on an average, in a given period of years. The frequency period will depend on the importance and cost of the structure. Suggested values are: 10 years for small, readily replaced structures such as diversion ditches and terraces; 25 years for small earth dams; and 50 years for larger earth dams or where failure would cause serious inconvenience. Larger structures, where cost of replacement is high, or life endangered, will be designed for larger run-off rates.

Volume of surface run-off is regarded from a different viewpoint. Here the designer is not interested in the exceptionally high values, but in the dependable average. The frequency with which the usable water supply can be allowed to fall below a required minimum depends on the purpose of the water, cost of construction of reservoir, economic value of water, and hydrologic factors. Krimgold (9) defines a dependable water supply for the Claypan Prairies (Illinois, Indiana, Iowa, Missouri, Kansas, and Oklahoma) as being one which, on the average, can be depended upon 80, 90, or 96 per cent of the time. Data or information available to the engineer to assist him in estimating the probable rate or volume of surface run-off for a given watershed in the Southern Prairie region are not available in readily usable form. Design is often based on individual experience.

This article will discuss some of the aspects of the run-off problem for small watersheds, as commonly encountered in soil and water conservation work. An analysis of Dominion Water and Power Bureau records for streams of the Southern Prairies will be presented in an effort to bring existing data into a more usable and applicable form for the above purpose.

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FACTORS INFLUENCING RUN-OFF

Precipitation falling on the land surface is disposed of as:

- (1) Surface run-off to streams, lakes or ponds.
- (2) Direct evaporation.
- (3) Transpiration.
- (4) Deep seepage beyond the range of plant roots. This water maintains the ground water supply, and may reappear as springs.
- (5) Soil moisture accretion.

Surface run-off will occur when rain or melting snow is releasing water on the land surface faster than the earth can absorb it. The greater portion of the run-off volume, and most of the peak run-off rates, in this region, are the result of melting snow. Intense summer rains on occasion cause peak flood rates on watersheds of less than two square miles. These intense rains are usually local in extent, and hence affect a small watershed of a few acres much more than one of several square miles. Peak flood rates on large watersheds, due to extended, excessive summer rainfall, occur rarely in this region.

There are many factors that influence the rate and volume of run-off, and their inter-relation is so complex that, with the present meagre knowledge, it is impossible to evaluate them all in a simple expression. An expression giving run-off rate in terms of all the factors concerned would be attractive theoretically, but practically might have little value, and is not necessary.

Some of the factors affecting rate and volume of run-off will be briefly listed:

- (1) *Watershed area.* The rate of run-off per unit area decreases with increasing watershed size. The volume of run-off per unit area may increase or decrease, depending on other watershed characteristics.
- (2) *Climate.*
 - (a) Type and frequency of excess precipitation.
 - (b) Temperature. Areas where temperatures are below freezing for a portion of the year will have different run-off characteristics from areas where this is not the case. Temperature affects the rate of melting of snow and evaporation.
 - (c) Wind and humidity, combined with temperature, affect the evaporation rate and hence the run-off.
 - (d) Special local conditions, such as the chinook wind in South Alberta and Southwestern Saskatchewan.
- (3) *Watershed shape.* A compact shaped drainage area will have a higher run-off rate than a long, narrow area of similar size.
- (4) *Watershed exposure,* i.e., north or south facing.
- (5) *Soil type.* The infiltration rate and capacity will influence the rate and volume of run-off.
- (6) *Vegetative cover and land use.*
- (7) *Average slope of the area.*
- (8) *Topography and drainage pattern.*

The Southern Prairies are characterized by long, cold winters, and hot, often dry, summers. The total average precipitation is low, varying from a low of about 10 inches in Southeastern Alberta to a high of 16 to 20 inches in Southern Manitoba. Approximately one-quarter of this total may fall from November to March, largely as snow. June is normally the month of highest rainfall, with July and May following in order.

The spring thaw, occurring in the latter half of March or the first half of April, will normally produce some run-off, and the larger watercourses normally have their yearly peak run-off rate at this time. The smaller watercourses and coulees cease to flow as soon as the snow has gone, and only large rivers such as the Souris, or spring-fed streams as in the Cypress Hills continue to flow much after the end of June, except in years of abnormal precipitation.

The yearly peak rate and volume of run-off vary greatly from year to year, particularly on small watersheds. There are occasional zero values, and also the occasional very high run-off. Intense summer rains on occasion will cause a peak flow rate on small watersheds.

The run-off volume, or depth, is low, normal values ranging from a high of 3 inches for some streams of the Cypress Hills to less than 0.1 inch for the Souris River. The run-off volume varies greatly from year to year, and several consecutive years of subnormal run-off may occur quite frequently.

PRESENT STATUS OF THE PROBLEM

The Dominion Water and Power Bureau and its predecessors have maintained gauging stations, and secured run-off data for most of the larger rivers and streams in the Southern Prairies, starting in 1908. The records for individual streams vary greatly in length and completeness. The streams gauged are mainly those with larger drainage areas of from 15 to several thousand square miles, and the results therefore are not directly applicable to small drainage areas.

Many empirical formulae have been developed in the past for determining run-off rate. Most of these formulae were developed for a specific area, and great caution must be used in applying any such formula outside the conditions for which it was developed. The "Handbook of Applied Hydraulics" (3) lists 45 such formulae.

The United States Soil Conservation Service, and related agencies, have done much work in obtaining run-off data for small watersheds at various places within the United States. Krimgold (5, 6) deals at some length with the various factors and problems.

The "Rational Formula" has been used very considerably in the past in the United States for estimating the run-off rate from small agricultural areas. It takes the form: $Q = CIA$

where Q = peak run-off rate in c.f.s.

C = a run-off coefficient.

I = rainfall intensity in in./hr.

A = drainage area in acres.

Ramser (11, 12) was the first to apply this formula to agricultural areas, and to obtain values of the coefficient "C". The value of "I" depends on the location of the watershed, the estimated time of concentration and the frequency period. Values are selected from charts as in references (2, 10, 13). The value of the coefficient "C" depends on watershed slope, cover, soil texture, and many other factors, and is an estimate only.

Results of actual run-off measurement on small watersheds, and use of the above formula over a period of years, brought the realization that the many factors affecting run-off rate cannot be expressed satisfactorily in a single coefficient.

The method now coming into use is to derive run-off curves for different frequency periods from analysis of actual run-off data. A given curve will apply only to the area and conditions for which it was derived. As more run-off data become available, more and more of the country can be accurately covered. Krimgold (7, 8) expresses this trend of development. The method of presenting results from this method is well illustrated in reference (9).

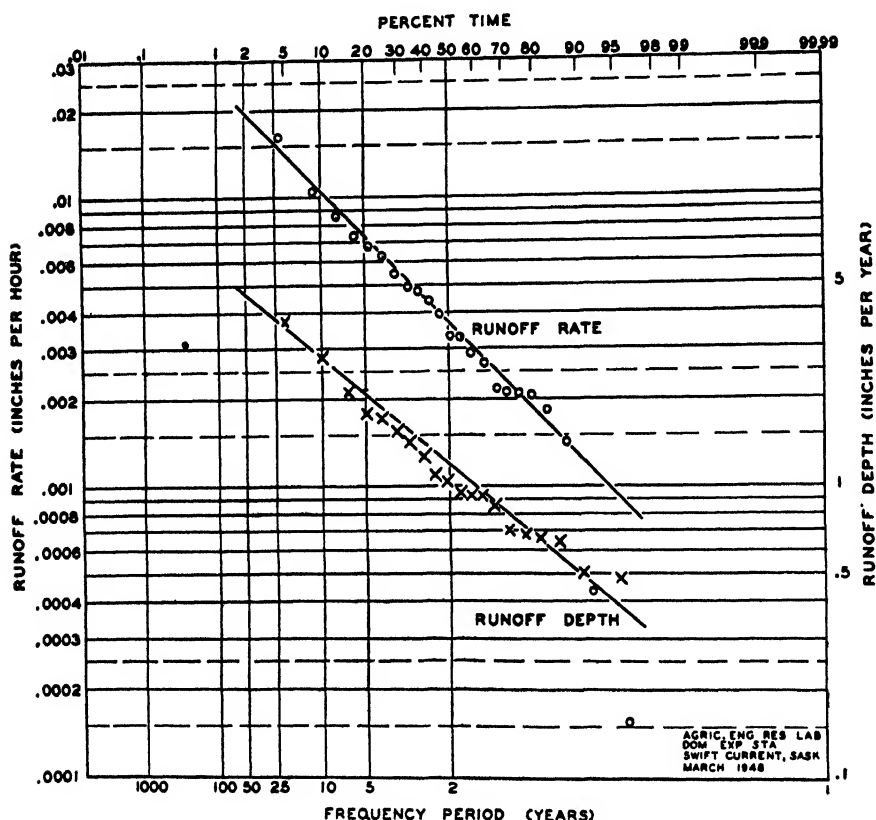


FIGURE 1. Run-off rate and depth frequency plotting for the Frenchman River at Eastend. (See Stream No. 23 in Table 1).

The Rational Formula has been discussed at some length due to its wide use in the United States. It applies to run-off from intense rain only, and does not apply to melting snow. There are few, if any, rainfall intensity records for Western Canada, so its use for the prairies is eliminated, except possibly in areas adjacent to the United States border where their data (13) might be applied.

The point which it is wished to emphasize is that, due to the limitations of the Rational Formula and the present lack of supporting data, no attempt should be made to apply it to the Prairie Region. Effort should be directed toward securing reliable run-off data to which probability analysis can be applied.

The Fuller Formula (4) has had some popularity. It was developed from records on United States rivers, largely those draining the more humid regions. The statement is made that streams having a "flashy" run-off characteristic, as in the more arid regions of the interior of the continent, do not fit the formula, and that the formula is not intended to be applied to those conditions.

The Meyer Formula (2, 3) has been developed from a study of all the principal streams and rivers in the United States.

The formula is of the form: $Q = qM = 100 pM^{0.5}$

where Q = peak discharge, c.f.s.

q = peak discharge, c.f.s. per square mile.

M = drainage area, square mile.

p = the Meyer rating of the stream.

For areas under four square miles, it is suggested the formula change to: $Q = qM = 50 pM$ or the run-off rate now varies as the first power of the area rather than the one-half power. Values of " p " are given for various streams on a map of the United States. The statement is made that the formula should be applied with caution for areas under 25 square miles. It will be noted that " Q " is the maximum, or limiting flood, and no mention is made of frequency.

The preceding has briefly discussed a few of the many run-off rate formulae.

ANALYSIS OF RUN-OFF RECORDS FOR STREAMS OF THE SOUTHERN PRAIRIES

The soil and water conservation studies, conducted at the Agricultural Engineering Research Laboratory, Swift Current, Saskatchewan, over the past ten years have revealed an acute need for more specific data on the rate and volume of run-off.

A beginning has been made on setting up run-off stations to measure the rate and volume of run-off from small agricultural areas in this region. However, some years must pass before sufficient data can be gathered to be of value. An attempt has been made to utilize existing stream flow data for use in the interval.

The run-off records of the Dominion Water and Power Bureau on streams of the Southern Prairies were analysed as described below. Only streams rising in this area were used; mountain streams and those with many lakes were excluded.

TABLE 1.—SUMMARY OF STREAM FLOW RECORDS FOR THE SOUTHERN PRAIRIE REGION

No.	Stream	Location of gauging station	Drainage area (sq. mi.)	Period of record	Run-off rate (in./hr.)—frequency in years*				Maximum discharge		Av. yearly run-off		Run-off vol.—Frequency**			
					2-year	10-year	25-year	50-year	In./hr.	Date	In.	Ac. ft./sq. mi.	Depth, in.	Ac. ft./sq. mi.	Depth, in.	Ac. ft./sq. mi.
1	Swift Current Creek	At Swift Current 5HD ₇	1160	1910-40	0.0013	0.0054	0.009	0.0127	0.00842	10 Apr. 17	0.92	49.0	0.82	43.8	0.48	25.6
2	Swift Current Creek	Sinclair's Ranch (Lower Sta.) 5HD ₃	366	1910-27 and 1936	0.0019	0.0042	0.0057	—	0.00394	Apr. 22	1.24	66.0	1.14	60.8	0.70	37.3
3	Swift Current Creek	Sinclair's Ranch (Upper Sta.)	172	1910-16	—	—	—	—	0.00227	8 Apr. 13	—	—	—	—	—	—
4	Swift Current Creek	Pollock's Ranch	16	1909-16	0.0015	0.0075	—	—	0.00543	17 June 16	1.17	62.2	1.12	59.8	0.64	34.1
5	Bone Creek	Lewis' Ranch	17	1910-16	—	—	—	—	0.0137	11 Apr. 12	—	—	—	—	—	—
6	Jones Coulee	Stearns' Ranch	23	1912-16	—	—	—	—	0.0027	20 June 15	—	—	—	—	—	—
7	Wood River	Near Gravelbourg 5JA ₁	2150	1918-25 and 1936	0.00074	0.0023	—	—	0.00655	30 Mar. 25	0.32	17.0	0.24	12.8	0.10	5.3
8	Notukeu Creek	Near Vanguard 5JB ₁	1421	1915-25, 1936 and 1940	0.0022	0.0045	—	—	0.0041	16 Apr. 40	0.42	22.4	0.33	17.6	0.15	8.0
9	Wiwa Creek	Near Gravelbourg	600	1921-25 and 1936	—	—	—	—	0.00413	29 Mar. 25	0.28	15.2	—	—	—	—
10	Moose Mountain Creek	At Oxbow, Sask. 5ND ₂	1900	1914-17, 1933-43	0.00026	0.0012	0.0021	0.003	0.00109	25 Mar. 39	0.13	6.9	0.09	4.8	0.031	1.7
11	Long Creek	Near Estevan	2240	1912-23,	0.00033	0.0012	0.0019	0.0026	0.00199	31 Mar. 43	0.137	7.3	0.115	6.1	0.030	1.6
12	Souris River	Glen Ewen, Sask.	6220	1912-17	—	—	—	—	0.00042	24 Apr. 16	—	—	—	—	—	—
13	Souris River	Melita, Man. 5NF ₁	19937	1912-22 and 1936	0.00009	80.00026	0.00037	—	0.000294	23 Apr. 16	0.108	5.7	0.1	5.3	0.052	2.8
14	Souris River	Estevan, Sask. 5NB ₃ , 5NB ₄	4760	1911-23, 1923-43	0.00029	0.001	0.0014	0.0019	0.00156	6 Apr. 43	0.095	5.1	0.094	5.0	0.0054	0.29

42	Battle Creek	International Boundary. 11AB ₁ .	72*	1917-39*	0.0013	0.0056	0.0093	0.013	0.0068	Mar. 27	0.74	—	0.59	31.4	0.26	13.9
43	East Branch Battle Creek	International Boundary. 11AB ₂ .	98	1927-43	0.0033	0.0113	0.018	—	0.0113	21 Mar. 39	0.45	24.0	0.38	20.3	0.015	0.80
44	Lyons Coulee	International Boundary. 11AB ₂ .	47	1927-43	0.0065	0.022	0.035	—	0.0222	21 Apr. 40	0.8	42.7	0.64	34.2	0.06	3.2
45	Woodpile Coulee	International Boundary. 11AB ₁ .	70	1927-43	0.0052	0.027	0.049	—	0.0321	30 Mar. 43	0.71	37.9	0.62	33.1	0.055	2.9
46	Manyberries Creek	Near Manyberries 5AF ₁₀	137	1911-31, 1935-43	0.005	0.019	0.03	0.041	0.0239	20 Mar. 28	1.02	54.4	0.90	48.1	0.52	27.7
47	Irrigation Creek	Near Manyberries 5AF ₁	85	1916-30 and 1936	0.0017	0.006	0.0095	—	0.007	20 Mar. 28	0.32	17.2	0.25	13.3	0.036	1.9
48	Ketchum Creek	Near Manyberries 5AF ₇	74	1916-25 and 1936	0.0028	0.0098	0.0155	—	0.00915	25 Mar. 18	0.55	29.3	0.39	20.8	0.15	8.0
49	Canal Creek	Near Manyberries 5AF ₉	72	1917-25 and 1936	0.0024	0.0055	0.0074	—	0.0055	2 Apr. 17	0.48	25.7	0.39	20.8	0.19	10.1
50	Maynard Coulee	Near Onefour, Alta. 11AA _W	12	1925-30	—	—	—	—	0.00452	23 May 27	—	8.5	—	—	—	—
51	Lindsay Coulee	Near Onefour, Alta. 11AA _W	9	1925-30	—	—	—	—	0.0108	23 May 27	—	57.0	—	—	—	—
52	Raymond Coulee	5AF _W	28	1927-31 and 1936	—	—	—	—	0.0326	22 Mar. 28	—	—	—	—	—	—

* Diversion to and from Cypress Lake starting 1839.

** To convert to per cent of time multiply 1/Frequency X 100.

† Record Incomplete.

TABLE 1.—SUMMARY OF STREAM FLOW RECORDS FOR THE SOUTHERN PRAIRIE REGION—Concluded

No.	Stream	Location of gauging station	Drainage area (sq. mi.)	Period of record	Run-off rate (in./hr.)—frequency in years ^a				Maximum discharge		Av. yearly run-off	Run-off vol.—Frequency ^{bb}				
					2-year	10-year	25-year	50-year	In./hr	Date		Depth, in.	Ac. ft./ sq. mi.	Depth, in.	Ac. ft./ sq. mi.	1.25 year
53	Maple Creek	At Maple Creek	81	1909-19	0.0048	0.0094	0.012	—	0.0086	4 Apr. 12	1.28	68.0	1.18	53.0	0.66	35.2
54	Maple Creek	At Dixon's Ranch 5HA ₁	360	1916-30, 1933-39	0.0024	0.0068	0.0099	0.0127	0.0085	24 May 27	0.79	42.0	0.55	29.3	0.23	12.3
55	Gap Creek	At Small's Ranch	108	1909-16	0.0068	0.0142	—	—	0.0143	4 June 15	1.39	74.0	—	—	—	—
56	Gap Creek	Near Maple Creek	274	1911-15	—	—	—	—	0.0066	3 Apr. 15	—	51.0	—	—	0.78	41.6
57	McShane Creek	At Small's Ranch	28	1910-14	—	—	—	—	0.00393	8 Oct. 14	—	15.0	—	—	—	—
58	Bridge Creek	At Gull Lake 5HA ₂	213	1911-22	0.00044	0.0018	—	—	0.0016	13 Apr. 17	0.27	14.4	0.095	5.1	0.018	0.96
59	Bridge Creek	At Skull Creek	15	1910-14	—	—	—	—	0.00455	14 Mar. 14	—	—	—	—	—	—
60	Bridge Creek	At Raymond's Ranch	6	1911-16	—	—	—	—	0.0175	8 July 15	—	118.0	—	—	—	—
61	Skull Creek	Near Skull Creek P.O.	32	1909-14	—	—	—	—	0.0274	4 May 09	—	94.0	—	—	—	—
62	Skull Creek	At Doyle's Ranch 5HA ₂	20	1911-22	0.011	0.05	0.087	—	0.0622	20 Apr. 20	2.96	160.0	2.65	141.0	1.60	85.3
63	Bear Creek	Unsworth's Ranch 5HA ₂	97	1909-30 and 1936	0.0048	0.0114	0.0158	0.0192	0.0167	22 Apr. 22	1.9	101.0	1.7	90.7	1.03	54.9
64	Piapot Creek	Cumberland's Ranch	51	1909-19 and 1936	0.0021	0.012	0.023	—	0.0168	21 June 09	1.11	59.5	0.84	44.8	0.35	18.7
65	Hay Creek	Hay Creek School	22	1911-21	0.0024	0.0075	0.0115	—	0.00845	17 Mar. 18	1.08	58.0	0.85	45.3	0.35	18.7
66	Boulder Creek	Young's Ranch near Walsh	104	1911-19	0.0019	0.0086	0.015	—	0.0067	9 Apr. 17	0.64	34.0	0.35	18.7	0.11	5.9
67	McKay Creek	At Walsh	200	1911-19	0.0029	0.0125	0.0215	—	0.0112	11 June 16	0.95	51.0	0.64	34.1	0.215	11.4
68	East McKay, McKay Creek	Grant's Ranch	77	1912-14	—	—	—	—	0.0228	3 Apr. 12	—	—	—	—	—	—

* Frequency in years based on 10-year average of annual run-off rates.

** Frequency based on 10-year average of annual run-off rates.

			1911-30, 1935-43	0.0042	0.0074	0.0091	0.0105	0.0104	22 Apr. 22	0.88	47.0	0.72	38.3	0.25	13.3
69	Ross Creek	At Irvine 5AH ₂	233												
70	Bullshead Creek	Burton's Ranch 5AH ₁₂	143	0.0028	0.0147	0.027	—	0.0243	10 July 23	0.9	48.0	0.77	41.1	0.38	20.3
71	Seven Persons Creek	At Medicine Hat 5AH ₁	744	0.00082	0.0027	0.0045	0.0057	0.0051	21 Mar. 18	0.33	17.6	0.23	12.3	0.025	1.3
72	Seven Persons Creek	At Seven Persons 5AH ₁₂	445	0.0008	0.0042	0.0077	—	0.00404	22 Mar. 28	0.32	16.0	0.18	9.6	0.053	2.8
73	Paradise Creek	Near Seven Persons 5AH ₁₂	112	0.0015	0.0115	0.024	—	0.0148	25 Mar. 25	0.41	22.0	0.26	13.9	0.082	4.4
74	Moose Jaw Creek*	McCarthy's Farm 5JE ₁	1960	0.00036	0.0024	0.0048	—	0.0024	14 Apr. 27	0.26	13.8	0.14	7.5	0.034	1.8

** To convert to per cent of time multiply /1 Frequency X 100.

* Dams on Moose Jaw Creek influence the run-off.

TABLE 2.—VALUE OF "k" IN THE EXPRESSION $r = \frac{k}{A^{0.5}}$

	Frequency		
	10-Year	25-Year	50-Year
Normal	0.14	0.21	0.285
Maximum	0.26	0.4	0.6
Minimum	0.035	0.06	0.13

The yearly run-off data, including yearly peak flow rate and date and yearly run-off depth, were tabulated separately for each stream. The peak run-off rates were reduced to inches per hour, and the volume to inches depth. These values were tabulated in descending order of magnitude. The percentage of time that each value was equalled or exceeded was calculated from the formula

$$p = \frac{100n}{y + 1} \quad \text{where} \quad \begin{array}{l} p = \text{per cent} \\ n = \text{number of the occurrence} \\ y = \text{number of years of record} \end{array}$$

The percentage was plotted against the corresponding value for run-off rate and volume respectively on logarithmic probability paper as in Figure 1. From the resultant curves, values of run-off rate for 2-, 10-, 25-, and 50-year frequencies and for run-off depth and volume, were tabulated for each stream in Table 1.

This method allows the frequency of a given peak flow rate to be determined. An estimate of the flood rate to be anticipated for a frequency period longer than the period of record may be made by extending the graph. Judgment must be used as the further the extension, the less reliable is the result.

There were 56 streams where the record was of sufficient length to warrant estimating the 10-year rate; 47 streams for the 25-year rate, and 26 streams for the 50-year rate.

For each of the 10-, 25-, and 50-year frequency periods, the run-off rate was plotted against the corresponding watershed area for each stream on log-log paper.

The resulting group of points did not fall on a single line, but as a group definitely indicated a relation between run-off rate and watershed area. A line with a slope of minus one-half fitted the points well (as in the Meyer formula), and this was the slope used.

The unit run-off rate for a given frequency period may be expressed:

$$r = \frac{k}{A^{0.5}} \quad \text{where} \quad \begin{array}{l} r = \text{run-off rate, inches per hour.} \\ A = \text{watershed area, square mile.} \\ k = \text{a coefficient.} \end{array}$$

$$\text{or } Q = 646kA^{0.5} \quad \text{where } Q = \text{c.f.s.}$$

The points scatter from the above line, but examination showed that the more extreme variations could be logically accounted for, if the watershed characteristics were considered.

Maximum and minimum lines to encompass all points, and a normal line, all on the minus one-half slope, were drawn for each frequency period.

From this, three values of "k", a maximum, minimum, and a normal were found for each frequency period.

The normal values of "k" in Table 2 may be expressed in terms of the Frequency Period, T: $k = 0.05T^{0.444}$

The peak run-off rate expectancy may now be expressed in terms of the Frequency Period (T) and the Watershed Area (A):

$$Q = 32.3A^{0.5}T^{0.444}$$

where Q = flood flow in c.f.s. that will be equalled or exceeded, on an average, once in a period of T years.

A = watershed area in square miles.

T = frequency period in years.

A coefficient must now be applied to compensate for variations from the normal due to watershed characteristics and other factors.

$$Q = C(32.3A^{0.5}T^{0.444})$$

where C = a coefficient depending on watershed location and characteristics.

or

$$Q = C(1.3a^{0.5}T^{0.444})$$

where a = watershed area in acres.

Values of the coefficient "C" to apply to specific locations and conditions were selected from an examination of the points representing individual streams on the Run-off Rate Watershed Area plottings.

The Run-off Chart, Figure 2, gives the run-off rate to be anticipated in a 10-year period for various values of the run-off coefficient. Conversion factors are given to convert values from the chart to 25- or 50-year frequency periods.

This chart applies to that area bounded by the United States-Canadian boundary, St. Mary, Oldman, South Saskatchewan, Qu'Appelle, and Souris Rivers.

In order to illustrate the application of Table 3 and Figure 2 to the solution of a practical problem in water conservation, the following example is given: A water storage reservoir is to be constructed on a watershed northeast of Hodgeville, Saskatchewan, in Township 14, Range 7, West of the Third Meridian. It is found that this watershed is tributary to Wiwa Creek. A survey of the drainage area indicates that its area is approximately seven square miles. The characteristics of the watershed indicate that a Run-off Coefficient of $C = 1.0$ (Table 3) may be used with reasonable safety.

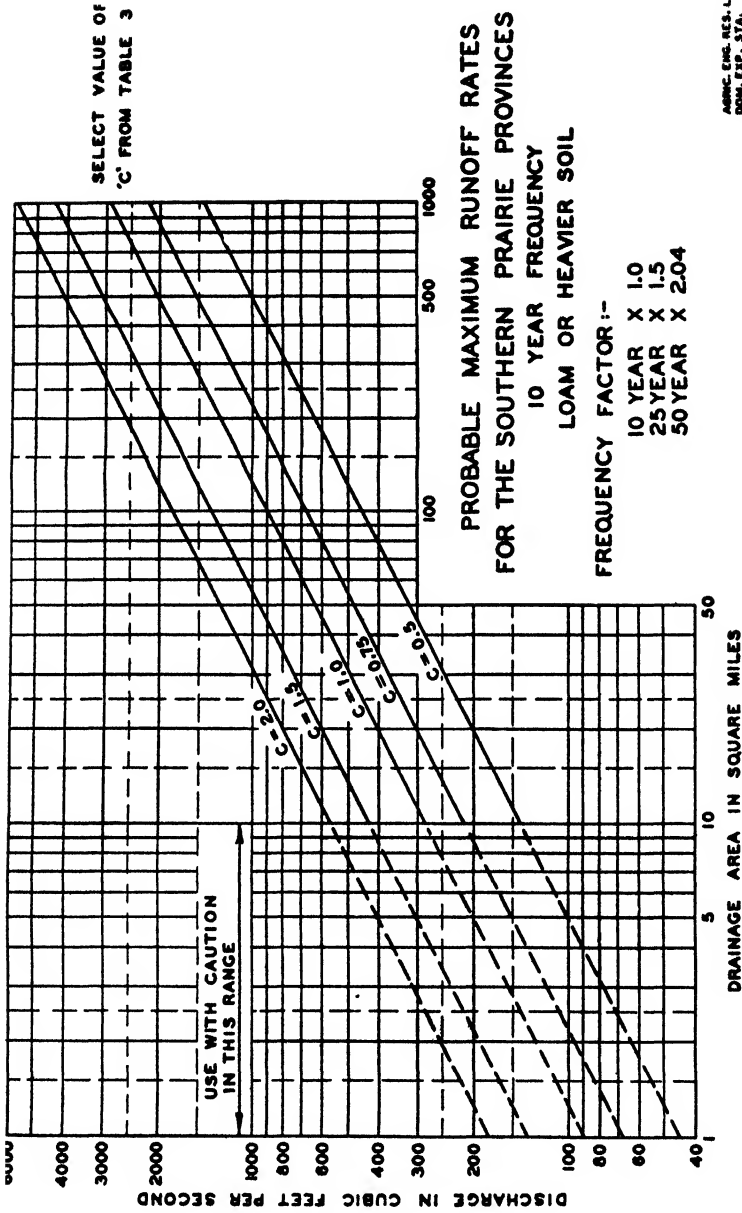


FIG. 2

TABLE 3.—VALUES OF RUN-OFF COEFFICIENT "C"

Value of "C"	Where applicable
2.0	The extreme rate. For steep, partially wooded watersheds in the higher elevations of the Cypress Hills, where conditions favour a very high run-off rate.
1.5	For fairly steep, hilly topography in the Chinook Area. Use for the area south of the Cypress Hills and Wood Mountain, and for the north slope of the Cypress Hills where conditions favour a high run-off rate.
1.0	The Normal Rate. This value could be used with reasonable safety where there are no factors indicating an exceptionally high or low rate. For level to moderately rolling topography, south of the Cypress Hills and Wood Mountain area. For moderately steep to hilly topography north and east of Cypress Hills. For moderately to strongly rolling topography in Southeastern Saskatchewan and Southwestern Manitoba.
0.75	For level to gently rolling topography north and east of the Cypress Hills and Wood Mountain area. For moderately rolling topography in Southeastern Saskatchewan and Southwestern Manitoba.
0.50	The practical lower limit. For level to gently rolling topography, with only fair surface drainage, in Southeastern Saskatchewan and Southwestern Manitoba.

In order to determine the spillway requirements of the dam, it is necessary to know the maximum probable Run-off Rate. Enter Figure 2 at a drainage area of seven square miles. Proceed vertically to the diagonal $C = 1.0$. Thence proceed horizontally to read the discharge rate of 240 c.f.s. This is the maximum probable discharge occurring once in ten years. A structure of this nature would probably be designed for a 25-year or 50-year frequency. The frequency factor for a 25-year maximum run-off is 1.5. Therefore, the spillway may be designed for a maximum discharge of $1.5 \times 240 = 360$ c.f.s., which will be equalled or exceeded once in every 25 years.

DISCUSSION

The following empirical formula has been proposed for the southern portion of the Prairie Provinces:

$$Q = C(32.3A^{0.5}T^{0.44})$$

Q is the peak flood in c.f.s. that may be anticipated to be equalled or exceeded, on an average, once in a period of T years.

C is the run-off coefficient, the value of which depends on the watershed characteristics and geographic location of the drainage area.

A is the watershed area in square miles.

T is the frequency period in years.

The designer must realize that a flood corresponding to a given frequency period may occur in any year, and that "T" years will not necessarily pass before the capacity of the structure is exceeded.

The above equation was derived from records of streams with drainage areas ranging from 15 to 24,000 square miles, and it is believed that reasonably reliable results may be obtained when the formula is applied within this range.

The length of record of the streams analysed does not warrant the use of a Frequency Period (T), greater than 50 years.

The gauging stations from which the records were obtained would be influenced, in some cases, by diversion or storage on the watershed above the station. This will tend to decrease the run-off rate and, therefore, the results presented here must be regarded as being somewhat low. On the other hand, the run-off depth values given may be regarded as conservative, and the actual run-off will be higher.

This range of application covers a large number of small water conservation projects, highway bridges and culverts, and the like.

There are many cases where the drainage area involved is less than 15 square miles. The above equation probably can be applied with fair results to drainage areas of five square miles or greater; however, no claim is made for the accuracy of the results until checked by actual experience.

The author does not know whether or not the formula is applicable to drainage areas of less than five square miles, and this must await checking by actual measurement. The Meyer Formula (2, 3) suggests that the relation change to a function of the first power of the area for watersheds under four square miles. This was tried but the results appeared low. The suggestion is that, if this formula be applied to small areas under five square miles, it be left in the form given for the larger areas. The result should be conservative.

The values for run-off depth in Table 1 may be used for estimating the volume of run-off from a given area by using the value from the table for a similar stream in the same area.

Run-off volume tends to decrease with increasing size of drainage area; hence for small areas, values in Table 1 are probably conservative.

The normal run-off depth values for the Souris River are very low, about 0.1 inch. The Souris is a large river, and the watershed area contains a considerable portion of indefinitely drained land. It is suggested that for small, fairly well drained areas in Southeastern Saskatchewan and South-western Manitoba run-off depths of three to four times the values for the Souris River might safely be used.

Run-off volume varies considerably for watersheds in the same general area. The values in Table 1 represent the run-off from fairly well drained watersheds with a loam or heavier soil. There are many areas of light textured soils where the normal run-off depth will range down to zero.

The normal, or two-year frequency, depth of run-off is always lower in value than the average. This is due to years of exceptionally high run-off unduly affecting the average. The normal value is the better for general use.

The run-off depth for the 1.25 year frequency, in Table 1, represents the depth that will be equalled or exceeded once, on an average, each 1.25 years, or 80 per cent of the time.

It is hoped that this paper may serve to consolidate existing data on run-off for the Southern Prairies, and prove helpful to those engaged in small soil and water conservation projects.

No doubt, there is much room for improvement in the estimation of run-off rates from small areas, but little can be done until data from small drainage areas are available.

One run-off measuring station, to record the run-off from a 140-acre watershed, was set up in 1947 at the Dominion Experimental Station, Swift Current, Saskatchewan. It is hoped that in the future more such stations may be set up throughout this region to secure records on the run-off from small areas.

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THE PROBLEM OF UNDERSIZE FRUIT IN KIEFFER PEAR ORCHARDS¹

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During the past twenty years the Kieffer pear (*P. pyrifolia* × *P. communis*) has assumed considerable importance in Ontario, where it is used mainly for commercial canning purposes. In most of the Kieffer orchards a proportion of the trees show premature autumnal leaf-colouration (September and October) and bear undersized fruits of poor quality. Observations in Ontario nurseries show that from 3 to 10 per cent of the Kieffer trees exhibit a similar early autumn leaf-colouration. The trees which have shown early autumn leaf-colouration in nursery and orchard are usually dwarfish and show higher than normal mortality in the orchard. At times the fruit is so small that its sale is almost impossible.

These abnormal trees appear to occur at random throughout an orchard. Where an entire orchard, or one or more portions of it, is bearing small fruit, the trouble is probably due to poor physical condition of the soil or to unfavourable nutrient level.

The observations and experiments in this paper are presented to show the factors involved in the problem and to suggest some means of avoidance and correction.

EXPERIMENTAL MATERIAL AND METHODS

Physical Measurements

Tree-size—The cross-sectional area of the trunk, computed from girth measurements, was used as an index of tree-size.

Fruit-size—Fruit size was determined by weighing a number of fruits and recording the average.

Swelling at the graft-union—The diameter of the stock and scion was recorded at a distance of 2 cm. both above and below the union. The diameter at the point of union was also recorded. The difference between the average of stock and scion diameters and the diameter at the union was used as a measure of the swelling at the union.

Obstruction to passage of water and carbohydrates at the union—The methods reported by Chang (6) were used. The suction applied was equivalent to 700 mm. of mercury.

Strength at union—The apparatus used to determine the strength of the graft-union is shown in Figure 1. In this figure, A was a fixed point, the distance from point A to point B was 4 inches, and the distance from point B to point C was 40 inches. Thus there was a tenfold leverage. V was the vise which held the graft-union firmly in place. Pressure was applied at point C. A pressure of one pound with the fruit pressure-tester gave a force of 10 pounds at the graft-union. This force was expressed in pounds required to break one sq. cm. of cross-sectional area at the graft-union.

¹ The laboratory work was done by the senior author in partial fulfilment of the requirements for the degree of Doctor of Philosophy at Michigan State College.

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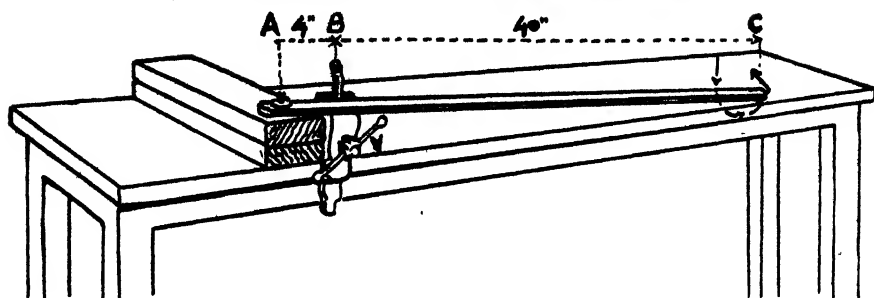


FIGURE 1. Diagram of breaking apparatus used for measuring the strength of the graft-unions.

Chemical Measurements

Total acidity—The acidity of the fruit was determined from a mixture of 100 gm. of fruit and 100 ml. of water thoroughly macerated in a Waring Blender. A 10-ml. sample was titrated with N/10 sodium hydroxide using phenolphthalein as indicator. The volume of sodium hydroxide required to neutralize this quantity was used as an index of acidity.

Total solids—The refractometer was used to measure the total solids in the pear juice.

Ascorbic acid—Ascorbic-acid content was measured by the method advocated by Lucas (8).

Tissue analysis—The material was air dried and ground. It was analysed for nitrogen, potassium, calcium, phosphorus, and total ash by Official Methods (3). Calculations were on the dry-weight basis.

Soil analysis—Spurway's methods were employed for determining the nutritional status of the soil (11).

RESULTS

Orchard Trees

Relationship between Leaf Colouration, Size of Tree, Size and Quality of Fruit

Green leaves were always associated with large trees and with large fruits of good quality, whereas red leaves were always associated with small trees and with small fruits of poor quality (Table 1). In comparison to the red-leaved trees, the green-leaved ones bore fruits with a significantly higher content of ascorbic acid. Total acidity, however, appeared to have no relationship to the colour of the foliage. Fruits from red-leaved trees were more gritty, i.e., contained more stone cells, were more astringent, and had a thicker hypodermal layer than fruits from green-leaved trees.

TABLE 1.—RELATIONSHIP BETWEEN AUTUMNAL LEAF COLOURATION, SIZE OF TREE, SIZE AND QUALITY OF FRUIT, VINELAND ORCHARDS, OCTOBER, 1948

Determination	Green-leaved trees, average of 4 trees*	Red-leaved trees, average of 4 trees*
Area of trunk x-section (sq. cm.)	200.1	105.2
Weight per fruit (gm.)	112.4	63.5
Total solids (%)	7.8	6.3
Total acid index	3.9	3.8
Ascorbic acid (mgm. per 100 gm.)	6.7	4.4

* For orchard and position see Table 2.

Chemical Analysis of Leaves

In both Ontario orchards the nitrogen and calcium contents were appreciably higher in green leaves than in red ones (Table 2). In the Troup orchard, the green leaves also contained more potassium, phosphorus and total ash than the red leaves. However, in the Culp orchard, this was not the case.

TABLE 2.—CHEMICAL ANALYSIS OF PEAR LEAVES, VINELAND ORCHARDS, OCTOBER, 1948*

Tree	Foliage colour	N	K	P	Ca	Total ash
		%	%	%	%	%
<i>Troup orchard</i>						
Row 1, Tree 19	Green	1.52	1.01	0.11	1.49	5.48
Row 1, Tree 18	Red	1.31	0.66	0.09	1.33	4.35
Row 2, Tree 14	Green	1.50	0.56	0.12	1.53	5.01
Row 2, Tree 13	Red	1.15	0.20	0.08	1.39	4.64
Row 5, Tree 17	Green	1.64	0.87	0.10	1.67	6.39
Row 5, Tree 16	Red	1.33	0.81	0.09	1.35	5.50
<i>Culp orchard</i>						
Row 3, Tree 17	Green	1.73	0.88	0.09	2.19	5.18
Row 3, Tree 16	Red	1.43	1.07	0.11	1.50	6.71

* Red- and green-leaved trees were paired, each individual tree representing an approximate average for its class in each orchard.

Soil Analysis

The soil beneath the trees listed in Table 2 was analysed for active and reserve nitrogen, phosphorus, potassium, and calcium, and for acidity (pH). There was no significant difference in the nutrition status of the soils upon which the two types of trees stood. Apparently premature autumn leaf-colouration was not associated with the lack of soil fertility in the cases under study.

Nursery Trees

Behaviour of Red-leaved Nursery Trees After Planting in the Orchard

The standard Kieffer trees with green foliage are usually larger in size in the nursery row than those with red foliage. Measurements of trunk cross-section, taken in 1948, of one-year trees on domestic seedlings gave the following averages: green leaf (70 trees), 1.86 sq. cm.; red leaf (8 trees), 1.38 sq. cm. This difference often widens year by year when these trees are transplanted to the orchard (Table 3). The size difference in the nursery may not seem to be very striking but the presence of these dwarf trees in the orchard may have an appreciable bearing on the total yield. There is a suggestion, however, that not all of the trees which show red leaves in the nursery develop into dwarf trees in the orchard; two of the six for which data are given in Table 3 are not dwarf trees. That these were the largest of the red-leaved trees in each lot at planting time is worthy of note. Some of the trees, originally red-leaved in the nursery, are now very dwarf trees but seldom show early leaf colouration or small fruit-size. In other words, uncongeniality with the rootstock is now being demonstrated in a different way. In a standard commercial orchard, however, these abnormal trees are still quite undesirable.

TABLE 3.—TRUNK CROSS-SECTIONAL AREA AND FOLIAGE-COLOUR RELATIONSHIPS OF 11 KIEFFER PEAR TREES IN NURSERY AND ORCHARD

Time of planting in orchard	Rootstock	Trunk cross-sectional areas (sq. cm.)			
		Green leaves		Red leaves	
		At planting	1948	At planting	1948
Fall, 1939	Fr. pear	2.5	28.7	1.3	8.1
		2.0	37.8	1.8	12.2
		2.5	47.4	2.3	45.8
Fall, 1943	Kieffer seedlings	1.3	38.9	0.8	18.6
		5.3	24.4	1.3	39.2
		—	—	0.8	23.5

Chemical Analysis of Tops (including leaves)

The woody tops and leaves of the green-leaved Kieffer trees had an appreciably higher content of nitrogen and calcium than those of the red-leaved trees (Table 4). This is the same relationship as found in orchard trees. Regarding potassium, the woody tops of the red-leaved trees had a higher content than the green-leaved trees but no such trend was found in the leaves. The woody tops of the green-leaved trees had less ash and phosphorus than the tops of the red-leaved trees but the green leaves contained more phosphorus and ash than the red ones. The leaves had a much higher percentage of total ash than the tops in both types of tree.

TABLE 4.—CHEMICAL ANALYSIS OF WOODY TOPS AND LEAVES OF KIEFFER PEAR TREES IN THE NURSERY ROW

Number of trees analysed	Foliage colour in October	Portion of tree	N	K	P	Ca	Total ash
			%	%	%	%	%
Lot 1	Green	Tops	1.52	0.72	0.08	0.37	2.04
		Leaves	1.64	1.85	0.15	1.48	7.47
	Red	Tops	1.15	0.79	0.11	0.33	3.03
		Leaves	1.31	1.35	0.10	0.70	4.80
Lot 2	Green	Tops	1.50	0.47	0.08	0.52	2.17
		Leaves	1.73	1.05	0.14	1.55	7.13
	Red	Tops	1.33	0.80	0.11	0.37	3.05
		Leaves	1.43	1.39	0.12	0.76	5.24

Swelling at the Graft-union

The Kieffer trees with red foliage had greater swelling at the union than those with normal green foliage (Table 5). In red-leaved trees the graft-unions were rough in outer appearance while in green-leaved trees they were smooth and symmetrical. Bradford and Sitton (5) and Amos *et al.* (1) found that there was no direct correlation between degree of swelling at the union and congeniality. Other writers on stock-scion

compatibility, however, report an association between swelling at the union and lack of congeniality (2). Proebsting (10) noted interference with translocation across certain graft-unions and found a relation to swelling at the union. In the present studies a similar relationship was found.

TABLE 5.—MEASUREMENT OF SWELLING AT THE GRAFT-UNION BETWEEN KIEFFER AND DOMESTIC PEAR SEEDLINGS

Combination	Number of trees	Age of top	Average diameter of trunk 2 cm. above and below union (A)	Diameter of union (B)	Swelling at union (B minus A)
			cm.	cm.	cm.
<i>Lot 1—Fall, 1946</i>					
Kieffer (green leaf)	3	2 years	1.30	1.72	0.42
Kieffer (red leaf)	3	2 years	1.46	2.13	0.67
<i>Lot 2—Fall, 1947</i>					
Kieffer (green leaf)	4	2 years	1.81	2.26	0.45
Kieffer (red leaf)	4	2 years	1.61	2.18	0.57
<i>Lot 3—Fall, 1947</i>					
Kieffer (green leaf)	1	1 year	1.08	1.70	0.62
Kieffer (red leaf)	1	1 year	1.19	1.95	0.76

Obstruction to the Passage of Water in the Region of the Union

Less water passed through the red-leaved Kieffer graft-unions than through the green-leaved graft-unions (Table 6). Evidently, there is more resistance to the passage of water at the point of union in the red-leaved trees.

TABLE 6.—WATER CONDUCTIVITY THROUGH THE GRAFT-UNION

Combinations	Number of trees	Age of top	Mean cross-sectional area	Mean total water passed in one hour	Water passed per hour per sq. cm. cross-section
			sq. cm.	cc.	cc.
<i>Lot 1—Fall, 1946</i>					
Kieffer (green leaf)	3	2 years	1.33	56.5	42.5
Kieffer (red leaf)	3	2 years	1.69	35.9	21.5
<i>Lot 2—Fall, 1947</i>					
Kieffer (green leaf)	4	2 years	2.57	125.1	48.7
Kieffer (red leaf)	4	2 years	2.03	79.8	39.3
<i>Lot 3—Fall, 1947</i>					
Kieffer (green leaf)	1	1 year	0.95	35.2	37.0
Kieffer (red leaf)	1	1 year	1.00	28.7	28.7

Starch Accumulation at the Union

Red-leaved Kieffer trees (nursery) showed a deposit of starch above the union while green-leaved trees showed no such deposit. This indicates that the translocation of elaborated foods from the scion to the stock was not normal in the red-leaved trees. As a result, there was an accumulation of the products of photosynthesis (mostly carbohydrates) above the union. This condition is known to favour anthocyanin formation (9). The partial check to the passage of elaborated foods from scion to stock "starves" the stock which in turn dwarfs the scion.

Strength at Union and Nature of Fracture at the Graft-union

The graft-union of nursery trees having red foliage was weaker than that of trees with green foliage. The nature of the fracture at the union was moderate in the former case, and tearing in the latter (Table 7). The results with the red-leaved trees in this test were similar to those reported by Chang (6) for incompatible combinations.

TABLE 7.—STRENGTH AT UNION AND NATURE OF FRACTURE AT THE GRAFT-UNION

	Two-year Kieffer on domestic pear seedlings				
	Number of unions	Diameter at union	Total pressure required to break	Pressure to break per sq. cm. x-sec. area	Nature of fracture
		cm.	lb.	lb.	
Kieffer (green leaf)	3	1.72	163.0	70.8	Tearing
Kieffer (red leaf)	3	2.13	137.0	36.5	Moderate

DISCUSSION

These studies indicate that with the Kieffer pear the relationship between premature autumnal leaf colouration and small size of fruit is due to nutritional difficulties brought about by a poor graft-union.

The graft-unions of red-leaved Kieffer trees show a partial check to the passage of water and elaborated foods at the point of union. In contrast to the graft-unions of green-leaved trees, they are weaker and show greater swelling. In a congenial graft, water, mineral nutrients, and elaborated foods are freely exchanged between stock and scion. However, in uncongenial grafts there is a partial check to the passage of these materials in the regions of the union, which adversely affects the growth, cropping and longevity of the trees.

Experiments at Vineland Station show that these "off-type" pears are not due to differences in strain (7). Poor soil may result in early leaf colouration and undersize fruits but in the present investigations the soil-analysis figures did not furnish an explanation of the differences in behaviour found in the abnormal trees.

The deficiency of nitrogen and calcium encouraged anthocyanin formation in the leaves. Blank (4) reports that a decrease in nitrogen increases anthocyanin formation in barley kernels. Meyer and Anderson (9) agree that a deficiency of nitrogen favours anthocyanin formation. Results of the present investigations are in accord with the observations of these authors. Calcium had a close relation to nitrogen in the Kieffer leaves. As with nitrogen, a deficiency of calcium seems to encourage anthocyanin formation. Blank (4) says that Lundegardh found calcium deficiency in the tomato to be the cause of an increase in formation of anthocyanin pigment.

Two conditions tend to cause an accumulation of carbohydrates in the tops of the abnormal trees: (1) obstruction to the downward flow of carbohydrates at the union, and (2) resistance to the upward flow of nitrogen at the union, which limits the synthesis of amino acids from the carbohydrates. On the other hand, the early colouration of the foliage undoubtedly reduces the total carbohydrate supply in the tree through a reduction in photosynthesis.

Orchard experience suggests that Kieffer (*P. pyrifolia* \times *P. communis*) is not always congenial on French or domestic pear (*P. communis*) seedling rootstocks, nor does it always do well on its own seedlings (Table 3). A better rootstock for Kieffer would be desirable. Unless such a rootstock becomes available it may be wise to grow Kieffer on its own roots. Tukey (12) records the existence of such an orchard in the Hudson Valley, New York. The trees in this orchard are very healthy, productive and uniform. The chief possibility of getting Kieffer on its own roots is from cuttings. Considering the popularity of Kieffer pears with the canners, the growers should think seriously about using own-rooted Kieffer trees for future plantings. Unfortunately propagation of Kieffer from cuttings is not a commercial possibility in Ontario or in the north-eastern part of United States. Growers will probably have to get own-rooted Kieffer trees from Georgia or adjoining states where Kieffer can be propagated from cuttings.

At present the best plan appears to be the elimination of all small red-leaved Kieffer trees from the nursery. Because of the high fertility conditions common to nurseries, it is possible that some trees with poor unions will show little evidence of stress in the nursery, but in the orchard, under less favourable conditions, will show various signs of uncongeniality. Such trees should be replaced early in the life of the orchard.

CONCLUSIONS

Premature autumnal colouration of the leaves of the Kieffer pear, small size and poor quality of fruit, may be due to nutritional difficulties brought about by a poor graft-union. A low level of nitrogen and calcium seems to favour anthocyanin formation in Kieffer leaves.

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CONGENIALITY OF SOME PEAR VARIETIES ON QUINCE A¹

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Quince A (Angers) is the most common dwarfing rootstock for pears. Unfortunately there is frequent disagreement between scion and rootstock, varying in degree and nature with each variety. Bosc gives a poor take of buds and poor growth in the nursery but recovers its vigour in the orchard. Kieffer takes and grows well in the nursery but survives only a year or two in the orchard. The unions of Bartlett, Clapp Favorite, and Kieffer are weak, subject to breakage by wind in the nursery, or by handling at time of digging. On the other hand, Anjou, Duchess, Hardy, and Old Home seldom show signs of incompatibility, and the unions seem reasonably strong. These varieties are now being tested at Vineland as intermediate stocks for Bartlett and Bosc.

REVIEW OF LITERATURE

Argles (1) has given a very complete summary of investigations on this subject. His Table 1 (Appendix) gives the results with many varieties on quince stocks in various parts of the world and shows some instances of variability in behaviour from one country to another.

METHODS AND MATERIALS

The methods used in this investigation were the same as already reported in another paper (3) and were based on extensive experimental work on incompatibility by Chang (2). Part of the nursery trees used were grown at the Ontario Horticultural Experiment Station, and part in Ontario nurseries. All were No. 1 trees representative of the particular combinations and ages.

TABLE 1.—PERCENTAGE INCREASE IN DIAMETER AT THE UNION COMPARED TO THE AVERAGE 2 CM. ABOVE AND BELOW IT*

Age of top	Scion variety	Quince A rootstocks	Domestic pear seedling rootstocks
<i>One-year</i>	Bartlett	57	32
	Clapp Favorite	53	21
	Old Home	48	
	Hardy	36	
	Orange quince	25	
<i>Two-year</i>	Kieffer	60	
	Anjou	33	
	Old Home	31	
	Hardy	30	

* Six trees of each combination.

¹ Adapted from a thesis submitted by the senior author in partial fulfilment of the requirements for the Degree of Master of Science in Agriculture at the Ontario Agricultural College.

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RESULTS

Swelling at Union

Pear on quince results in an appreciable swelling at the union compared with pear on domestic pear seedlings and with Orange quince on Quince A (Table 1). The varieties considered least congenial with the quince, viz., Kieffer, Bartlett, and Clapp Favorite show greater swelling at the union than the more congenial varieties, Old Home, Hardy, and Anjou.

Rate of Vacuum-induced Water Flow Through the Union

For this test, the trees were freshly cut off 4 cm. above the union and the same distance below it. Through this 8-cm. section water was drawn by a force equivalent to 700 mm. of mercury. Measurements of water flow were commenced 10 minutes after the apparatus was connected and readings were taken every hour for four hours. Results were expressed on the basis of 1 sq. cm. of cross-section, using the average of diameters 2 cm. above and below the union (Table 2).

It is clear that the unions between Bartlett, Clapp Favorite, and Kieffer as scions and Quince A as rootstock offer considerable resistance to an "artificial" flow of water in the laboratory. The reason for the slow flow of water through the Orange quince union with Quince A is not clear though there is a possibility that the Orange quince scion wood offers high resistance. There was less resistance to water flow through the unions of Old Home and Hardy on Quince A than through the unions of Bartlett and Clapp Favorite on domestic pear seedlings.

TABLE 2.—RATE OF VACUUM-INDUCED WATER FLOW THROUGH THE UNION*

Age of top	Scion variety	Quince A rootstocks	Domestic pear seedling rootstocks
		cc. per hour per sq. cm.	
<i>One-year</i>	Bartlett	17.2	42.8
	Clapp Favorite	10.5	41.8
	Old Home	55.9	
	Hardy	61.1	
	Orange quince	20.8	
<i>Two-year</i>	Kieffer	13.1	
	Anjou	40.7	
	Old Home	44.7	
	Hardy	54.7	

* Six trees of each combination.

Starch Accumulations at the Union

Iodine tests on longitudinal sections through the unions of dormant trees showed heavy deposits of starch above the unions of Bartlett, Clapp Favorite, and Kieffer on Quince A but no such accumulation in Bartlett and Clapp Favorite on domestic pear seedlings, or in Old Home, Hardy, or Anjou on Quince A. There was some accumulation of starch *below* the unions of Orange quince on Quince A.

Bark and Wood Continuity at the Union

Kieffer on Quince A showed discontinuity in both wood and bark. Sometimes Bartlett and Clapp Favorite were in this class also, but more often the bark was continuous and wood discontinuous. All of these combinations showed a more or less prominent brown layer at the union. In Bartlett and Clapp Favorite on domestic pear seedlings, and in Old Home, Hardy, and Anjou on Quince A, both bark and wood were continuous and there was no brown line at the union.

Strength at Union and Nature of Fracture

It took over three times as much force to break the unions of Bartlett and Clapp Favorite on domestic pear seedlings as was required to break the relatively weak unions that these varieties made with Quince A (Table 3). On the other hand, Old Home, Hardy, and Anjou made such strong unions with Quince A that breakage occurred first on the stem of the rootstock just below the union.

TABLE 3.—STRENGTH AT UNION (LB. PER SQ. CM.) AND NATURE OF FRACTURE*

Age of top	Scion variety	Quince A rootstocks	Domestic pear seedling rootstocks	Nature of fracture
<i>One-year</i>	Bartlett	27	—	Smooth Tearing Smooth Tearing
	Bartlett	—	83	
	Clapp Favorite	25	—	
	Clapp Favorite	—	84	
	Old Home	broken below, not at union	—	
	Hardy	broken below, not at union	—	
	Orange quince	90	—	
<i>Two-year</i>	Kieffer	26	—	Smooth
	Anjou	broken below, not at union	—	
	Old Home	broken below, not at union	—	
	Hardy	broken below, not at union	—	

* Four trees of each combination.

DISCUSSION

All of the tests discussed in this paper show that Bartlett and Clapp Favorite are not congenial with the Quince A rootstock. This does not necessarily mean, however, that the trees will be short-lived. Given adequate continuous support in orchard or garden they may live for many years, but without support, intentional or otherwise, they are likely to break at the union when subjected to strong winds. Experience in Ontario has shown that Kieffer on Quince A will not survive for more than a few years in the orchard even when given adequate support. On the other hand, Old Home, Hardy, and Anjou appear to make strong unions with Quince A, so strong that one might be tempted to say supports are not required. However, lacking the assurance of tests under orchard conditions, it is probably desirable to give support also to these latter varieties. A union good in the nursery does not necessarily assure a continuously good union in the orchard.

SUMMARY

Bartlett, Clapp Favorite, and Kieffer are not congenial with Quince A rootstocks, their uncongeniality being demonstrated in the nursery by abnormal swelling at the union; by resistance to flow of water through the union; by starch accumulations above the union; by discontinuity of bark or wood tissues at the point of union; and by ease of breakage at the union. Old Home, Hardy, and Anjou seem to be reasonably congenial with the Quince A rootstock.

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SEEDLING AND CLONAL ROOTSTOCKS FOR PEARS

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In pear orchards, Kieffer particularly, there is often considerable variation in the time that the leaves of individual trees begin to take on the bright red and purple autumn colours. The same situation prevails in the nursery row where it is quite common to see, even as early as August, a small proportion of the trees beginning to show these high colours. By October, the colours of these same trees are very pronounced and defoliation may have commenced. These off-colour trees occur in blocks budded on French pear, American domestic pear, and Kieffer seedlings. Where the affected trees are concentrated in one or more areas of the orchard or nursery, unfavourable soil conditions are probably responsible but, where they are scattered throughout the whole planting, rootstock or graft-union relations appear to cause the trouble.

REVIEW OF LITERATURE

In 1933, Hatton (2) reported differences in autumn leaf colouration and defoliation between pear trees on several clonal rootstocks and Chang (1) gave premature autumn leaf-colouration and defoliation as signs of incompatibility. Randhawa *et al.* (3) found that these abnormalities in the Kieffer pear are often related to obstructions and weakness at the graft-union, and are associated with small size and poor quality of fruit in the orchard.

MATERIALS AND METHODS

In the fall of 1939, in the Experiment Station nursery the two- and three-year pear trees showing early leaf-colouration were marked. Beside each one, a normal green-leaved tree of the same variety was also marked. These trees were planted in pairs in November. At the same time and in the same row four one-year Kieffer (*P. pyrifolia* × *P. communis*) pear trees on each of six Malling clones were planted. Trunk measurements, foliage colour in the fall, and yield and size of fruit have been recorded annually for all of these trees.

RESULTS

Seedling Rootstocks

Out of six pairs of trees, two red-leaved ones are dead, two are much dwarfed, one is slightly dwarfed, and the remaining one is very vigorous—a larger tree than its mate, the green-leaved one (Table 1). This very vigorous red-leaved tree was also well above the average size for the variety in the nursery row.

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Fruit production from these trees has been very meagre, mostly a result of poor pollination conditions. The trees which had red leaves in the nursery in the fall have not had red leaves every year in the orchard; nor have the trees green-leaved in the nursery consistently had green leaves in the orchard; but there has been a greater tendency to remain in the nursery classification than otherwise. Apparently seasonal conditions play a large part in determining autumn leaf colouration.

As already suggested for the Kieffer variety (3) it seems very likely that a high proportion of the *small* red-leaved pear trees in the nursery will turn out to be dwarfs in the orchard. About the *large* ones there appears to be some doubt. In Kieffer, the red-leaved trees are sometimes as prevalent as ten per cent of the nursery block but much less common in the other varieties, probably around one per cent on the average. In standard orchards these dwarf trees represent a direct loss but, if the graft-union is reasonably strong and the tree well anchored, the seedling stock might have possibilities as a dwarfing clone provided it could be propagated readily.

TABLE 1.—RELATION BETWEEN AUTUMNAL LEAF COLOUR IN THE NURSERY AND PERFORMANCE IN THE ORCHARD

Varieties†	Green-leaved trees, area of trunk cross-sec.		Red-leaved trees, area of trunk cross-sec.	
	Nov. 1939*	Nov. 1948	Nov. 1939*	Nov. 1948
	sq. cm.	sq. cm.	sq. cm.	sq. cm.
Bartlett‡	—	20.4	—	Dead
Ovid	4.9	64.2	4.5	Dead
Bartlett	1.5	45.8	1.8	14.3
Dean of Summer	3.1	94.2	1.8	50.9
Selection 140119	2.0	33.1	1.5	29.9
Doy. G. Boucher	2.0	42.8	4.5	69.2

† All on French pear seedlings.

* Time of planting in the orchard.

‡ Planted November, 1940; no size record at planting time.

Clonal Rootstocks

The differences in growth and fruiting of Kieffer on several Malling pear (*Pyrus*) rootstocks at the end of the ninth year in the orchard lend support to the conclusion reached in the test reported above, viz., that seedling roots may result in variable orchard trees. All of the Malling pear rootstocks were selected from seedling collections of European source (2). At East Malling at the end of the sixth year in the orchard, Dr. Jules Guyot on C7 rootstock had made more than double the growth made on C4 rootstock, these two representing the extremes among 13 clones.

Of the six Malling pear clones in this test at Vineland, two are very dwarfing to the Kieffer variety, and depress the yield per tree considerably (Table 2). These trees would be considered quite undesirable for a standard orchard. At the end of nine years in the orchard the B1 rootstock has the highest yield but, year by year, C8 has had the best record on leaf-colour and fruit size. A more lengthy test with greater numbers of trees

and on various soil types will be necessary in order to give these rootstocks a proper rating. One of the two dwarfing rootstocks in this test, C3, proved to be incompatible with Dr. Jules Guyot in one test at the East Malling Station (2), but the other one, D3, gave trees of better than average vigour. On the other hand, B1, C8, and C4, which produced vigorous trees of Kieffer at Vineland, were among the four lowest in vigour rating at East Malling when the scion variety was Dr. Jules Guyot. This difference serves to point out the importance of testing new rootstocks with many varieties and under various climatic and soil conditions.

TABLE 2.—TREE-SIZE AND YIELD OF KIEFFER PEAR ON SEVERAL MALLING PEAR (PYRUS) ROOTSTOCKS

Rootstock	Number of trees	Area of trunk cross-section		Accumulated yield
		Nov. 1939*	Nov. 1948	
		sq. cm.	sq. cm.	kgm.
B3/1	4	1.4	55.5	16
B1	4	1.2	52.6	41
C8	4	1.3	45.8	29
C4	3†	0.7	36.1	20
C3	4	1.0	8.1	5
D3	3†	1.2	6.4	5

* Time of planting in the orchard.

† A fourth tree was injured by an implement.

† A fourth tree died in 1941.

SUMMARY

The early colouration of pear leaves on individual trees in the nursery is probably an indication of uncongeniality between rootstock and scion. Planted in the orchard, such trees may soon die, they may be much dwarfed, or they may outgrow the trouble to become standard trees. Two out of six Malling pear clones have given very dwarf Kieffer pear trees.

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AUTOMATIC VENTILATION OF COMMON STORAGE

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INTRODUCTION

The extent to which common storage is used is often not appreciated, partially because there are no reliable statistics which describe the contents or even the number of such storages. There is also some tendency to consider common storage as a crude arrangement for the preservation of fruits, vegetables and nursery stock. Familiarity with the storage method makes possible some general statements concerning its nature, its importance, its weaknesses and the possibility of improving its performance.

A common storage differs from a refrigerated storage only in the method used to establish a desirable temperature for the storage of any given commodity. The common storage depends to some extent on soil temperatures (if underground) but largely on ventilation with outside air for the maintenance of desired temperatures. Since summer temperatures are generally too high to exert a marked preservative effect, only fall harvested crops are placed in common storage.

The present importance of common storages in Canada is indicated by the fact that most of the winter supplies of potatoes, turnips, onions, beets, parsnips, cabbage, and carrots, together with considerable quantities of apples, are held in common storage. There are several thousand commercial growers of vegetables in Ontario alone, and probably most of them have some form of common storage. This type of storage is used extensively by growers of horticultural nursery stock. There is a tendency on the part of turnip waxers and potato growers to build large, centralized common storage structures, some of them supported by government subsidies. Thus the utilization of common storage does not appear to be lessening.

The obvious weakness of common storage is its dependence on natural temperatures. However, in the opinion of the authors, inefficient operation, resulting in inaccurate temperature control, is a highly important weakness during the normal operating season. Any improvement in common storage must be applicable to many hundreds of storages if the improvement is to result in a *general* effect on the stored fall crops.

Despite the general use of common storage the authors were not able to find a report covering adequately the potential performance of common storages and in particular no record is at hand to show performance under automatic controls.

For purposes of this paper, good performance means that full use is made of cool outside air and that the desired temperature is maintained accurately. The present report has as its objective a study of performance,

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in terms of storage temperature, in two storages of commercial size which are situated on the campus of the Ontario Agricultural College, Guelph, Ontario.

Several years of manual operation of the ventilation systems showed the need of automatic controls before a detailed study could be attempted. Then it was found necessary to develop a suitable differential thermostat as part of the ventilation system.

This report contains first, a general description of the ventilation system used here and a full description of the new thermostat, and second, an analysis of performance based on temperature data collected during 18 months, and an estimate of probable performance during the past 20 years.

AUTOMATIC VENTILATION SYSTEM

There are several possible methods of controlling ventilation. That which has performed well here is illustrated in diagrammatic form in Figure 1. Cool air is drawn through a duct past an automatic shutter to a space under a false, slatted floor. Warmer air is drawn from the room at a ceiling level by a fan and expelled past an automatic shutter in a duct leading to a short chimney. The fan is started by a differential thermostat when the outside air is slightly cooler than that inside. Ventilation proceeds until the outside and inside temperatures are nearly equalized or until a desired inside temperature is reached. In the former instance the differential thermostat breaks circuit and in the latter a cut-out thermostat operates. Air moves from floor to ceiling past the load and acquires heat which is delivered outside the building.

Auxiliary instruments include a small constant duty fan which circulates air in the storage to reduce the spatial differences in temperature associated with air stratification during periods of no ventilation. Connected with the air circulation system is a thermostat which controls a heat source for use in those storages where heat is known to be necessary.

All thermostats are sensitive enough that possible control of temperature is of the order of $\pm 1^\circ \text{F}$.

NEW DIFFERENTIAL THERMOSTAT

All the parts of the ventilation system are standard but we were unable to find a differential thermostat which met our requirements. The latter are: first, sensitivity to a temperature difference of approximately 1°F . or better; and second, that the instrument must not be permanently distorted when the outside sensitive element is exposed to a seasonal temperature range of from 105°F . to -40°F .

Three variations of a thermostat were tested, and although two of them are not perfected, they have inherent qualities which warrant their description. The third one performs well and is now being manufactured.

The three thermostats have in common two air containing systems consisting of two copper wafers, each connected by copper capillary tubing to a steel bulb. The systems are nearly equal in internal volume and a seal is made when the systems are at a temperature of about 25°F . (thus they are under positive pressure at the working range above 30°F .). One

bulb is affected by inside temperatures while the other is carried outside the storage. The latter is protected from direct sun radiation. The wafers are mounted in a rigid box with their inner faces joined by a rigid halter (in two types) so that motion of one wafer is transmitted to the other. In the remaining type, motion of the bellows is independent.

Type one is shown in Figure 2. A microswitch is pivoted on a wall of the box in such a manner that its final position is controlled by a screw and spring operating a lever arm extending from the microswitch. The microswitch is mounted within the halter but not in contact with it, except when its switch is depressed by motion of the halter in the direction of the wafer controlled by outside temperature. The microswitches used will carry 10 amperes at 110 volts, and thus directly control a fan motor of $\frac{1}{4}$ h.p., or a relay if more power is required. The first two models of type one were used successfully in our storages and they have the very considerable advantage of simplicity and ruggedness. The present difficulty came to light when duplicates were made. It was found that the wafers, as purchased, varied considerably in sensitivity and that the pressures necessary to operate the microswitches also varied. Sensitivity of some instruments was not better than 8° F. More uniform wafers (or bellows) and microswitches would erase the difficulty and make type one most desirable for common storage application.

Type two is shown in Figure 3. In it the wafers operate independently with one of them carrying an arm terminated by a carbon contact which completes circuit by pressure on a second carbon contact on the second wafer. Potentially, type two should be extremely sensitive but the models so far are affected by mechanical vibration. The electrical system in type 2 is similar to that in type 3.

Type three (Figure 4) is now in production. A rigid insulating rod extends through the halter and carries a carbon contact at its distal end. Circuit is made through a second carbon contact which is screw adjusted. Current is supplied from a transformer at 6.3 volts and circuit is completed through a few turns of heating wire wound about, but insulated from, a bent strip of aluminum metal. Heating increases the curvature of the aluminum metal and the resulting mechanical pressure positively operates the microswitch.

All three types are adjusted by placing both air bulbs in a container of cold water. When both bulbs are at the same temperature, the contacts are adjusted so that circuit is barely broken.

PERFORMANCE

The Storages

Both of the storages from which the data were obtained have dimensions approximately as follows: length, 20 feet; width, 20 feet; average height, 10 feet. They have flat roofs which slope from 11 feet at the front to 9 feet at the rear. Insulation is fill-type, 6 inches thick on walls and roof. Its theoretical insulating effect is similar to 4 inches of standard insulation. The latter thickness is used commonly when the storage temperature is between 32° F. and 40° F. The insulation was examined mid-way in the study and it was in satisfactory condition. The floors are not insulated.

EXPLANATION OF FIGURES 1 TO 5 (see opposite page)

FIGURE 1. Diagrammatic vertical section of a common storage. The arrows indicate direction of air flow.

- A—Air inlet duct fitted with an automatic shutter and a manual door.
- B—Load.
- C—False, slatted floor.
- D—Air circulation duct fitted with a fan and if necessary with a heater coil.
- E—Heat control thermostat.
- F—Cut-out thermostat in circuit with the ventilation fan.
- G—Differential thermostat.
- H—Air exit duct with fan, automatic shutter and manual door.
- K—Sun shade over exterior sensitive element of the differential thermostat.

FIGURE 2. Differential thermostat, type 1. Copper wafers, A, are 3 inches in diameter, connected by $\frac{1}{8}$ inch O.D. (or smaller) copper capillary tubing C, to air-bulbs, B, length of B, 7 inches, diameter 2 inches, steel tubing. Halter G connects the inner faces of wafers A. Top and lateral view of micro-switch D pivoted at E and position-adjusted at H. Movement of the halter G to the right makes circuit in the micro-switch.

FIGURE 3. Differential thermostat, type 2. Wafers A, tubing C, and air bulbs (not shown) are identical with those in type 1. Motion of wafers A is transmitted to two carbon contacts P, the upper contact is adjustable by screw H. Wafer A, left, is connected to the outside air bulb. A small electric current passes through points P with auxiliary arrangements identical to those in Figures 4 and 5.

FIGURE 4. Differential thermostat, type 3. Wafers A, air bulbs B, capillary tubing C and halter G are identical to those in type 1. A rigid plastic rod J is fastened to the halter G. Rod J carries a carbon contact P at its lower end. It completes circuit through a second carbon contact P which is adjustable by screw H. Its electrical circuit includes a transformer K, heating wire N, coiled about a bent aluminum strip M. Curvature of the aluminum strip is adjustable by screw H. The convex equator of the aluminum strip is in contact with the switch button of a micro-switch D. Strip M expands with heat when contacts P come together. Expansion of strip M operates the micro-switch.

FIGURE 5. Schematic wiring diagram for differential thermostats types 2 and 3 and their accessories. T¹ is an integral cut-out thermostat operated by room temperature. T² is the differential thermostat carrying a 6.3 volt current (0.5 amp.) from a transformer. The bent aluminum strip is shown in contact with the micro-switch S which operates the load L either directly or through a relay.

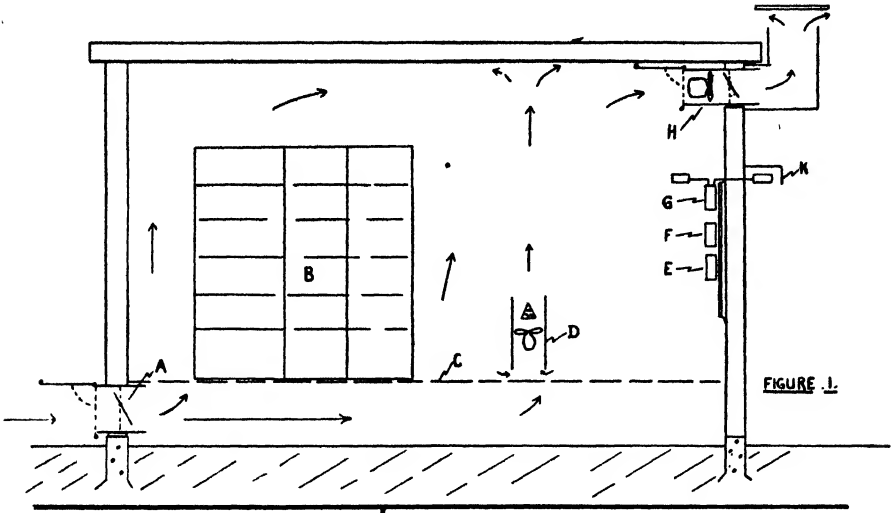


FIGURE 1.

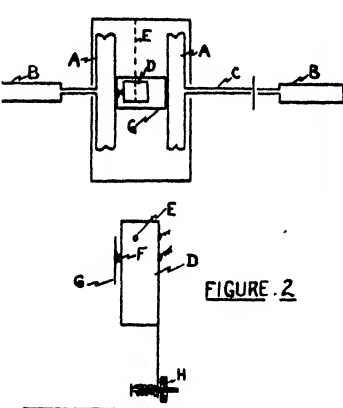


FIGURE 2.

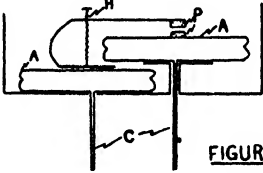


FIGURE 3.

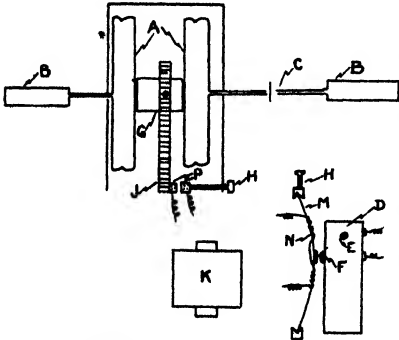


FIGURE 4.

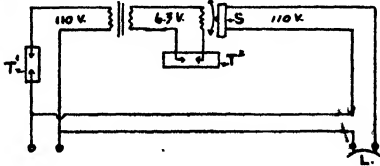


FIGURE 5.

FIGURES 1-5. (For explanation see opposite page)

One of the storages is of frame construction with heavy asphalted paper and $\frac{1}{2}$ inch lumber on both sides of the studdings and rafters. The roof is finished with heavy roofing paper. The sill rests upon cedar piles. This storage will be referred to as the frame storage.

The second storage has an outer bearing wall, 8 inches thick, of cinder-concrete blocks resting on a concrete foundation. The finish inside the insulation is painted, $\frac{1}{2}$ -inch lumber and the roof is similar to that on the frame storage. This storage will be referred to as the concrete storage.

Both storages may be described as adequate, cool storage structures. They have a high ratio of exposed surface area (1200 sq. ft.) to volume (4000 cu. ft.). They are isolated from other structures, but the concrete storage shades, from most of the sun's direct radiation, the southwest wall of the frame storage.

Other factors being equal, heat gains and losses are greater, proportionately, in smaller storages than in larger ones. The storages here are near minimum size for commercial use and the problem of heat gain and loss associated with surface-volume ratio is as serious as it is likely to be in commercial storages.

Required Storage Temperatures

The lowest temperature used in the commercial cool storage of horticultural materials is 30° F., but the most common is 32° F. Turnip, cabbage, carrot, beet, parsnip, onion, celery and many varieties of apples are stored at 32° F. Potato requires from 38° F. to 40° F. for long storage. The objective in common storage is to attain and hold those temperatures as soon as possible in the fall and to maintain them as late as possible in the spring. Obviously a temperature of 40° F. can be attained earlier and held later than a temperature of 32° F. The present work is related largely to the problem of reaching and holding 32° F.

Recorded Temperatures

The experimental data consist of the records made by a thermograph in each of the two storages. Each thermograph recorded outside and inside air temperatures on a 7-day chart. The thermographs were calibrated at the beginning of the study and checked subsequently by thermometers. On several occasions data were collected from maximum-minimum thermometers placed outside and inside the storages.

The measurements of temperature during mid-winter showed merely that the storages were being held at the desired temperature. The storages were empty, or nearly so, to allow maximum variations in temperature. During the winter of 1947-48 the ventilation system operated only briefly at isolated intervals of time. Because there was little or no biological heat produced in the storages the maintenance of temperature depended largely on a balancing of heat loss by an electric hot plate (1320 watts) operated by a thermostat.* The actual temperature was approximately 32° ± 1° F. in the winter season.

* Underground storages, held at 32° F., here require regular ventilation during winter to eliminate heat acquired from the soil.

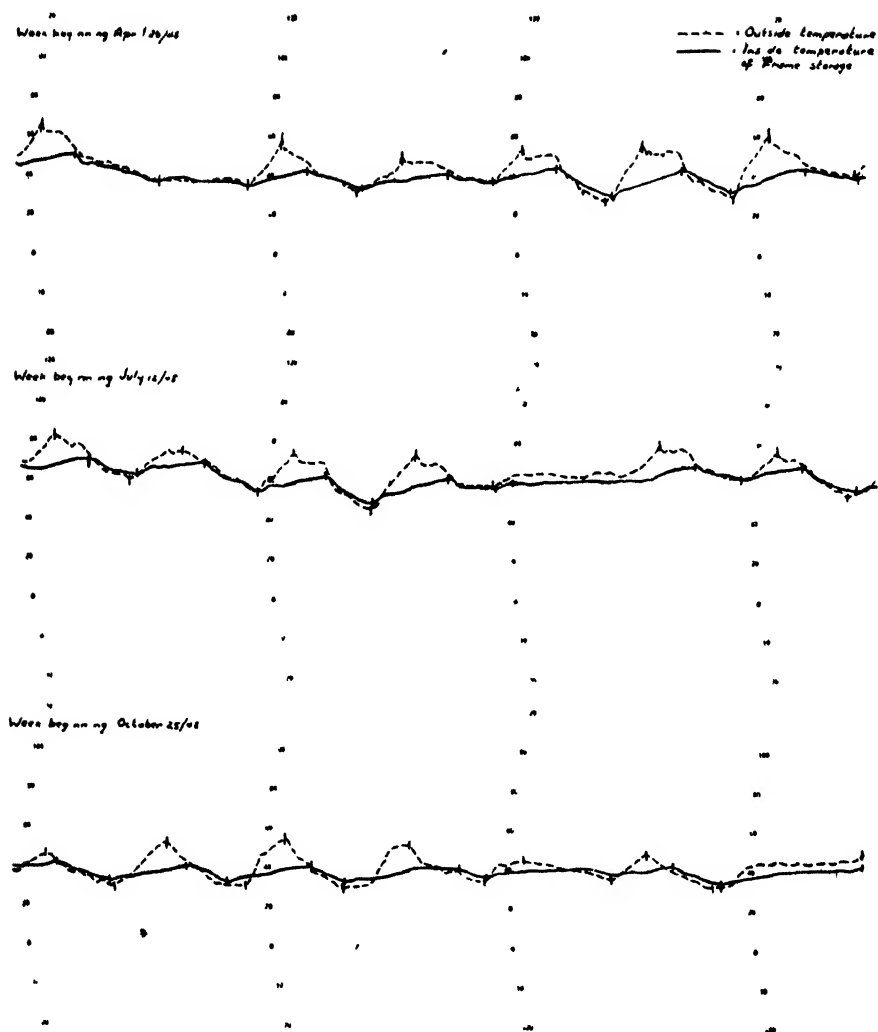


FIGURE 6. Temperature recordings, traced to synchronize time. Broken lines represent outside temperatures, continuous lines represent temperatures in the frame storage. Note that the inside temperature parallels the outside nightly drop in temperature. Vertical lines on the graphs represent principal trends as used in calculating time-temperature units.

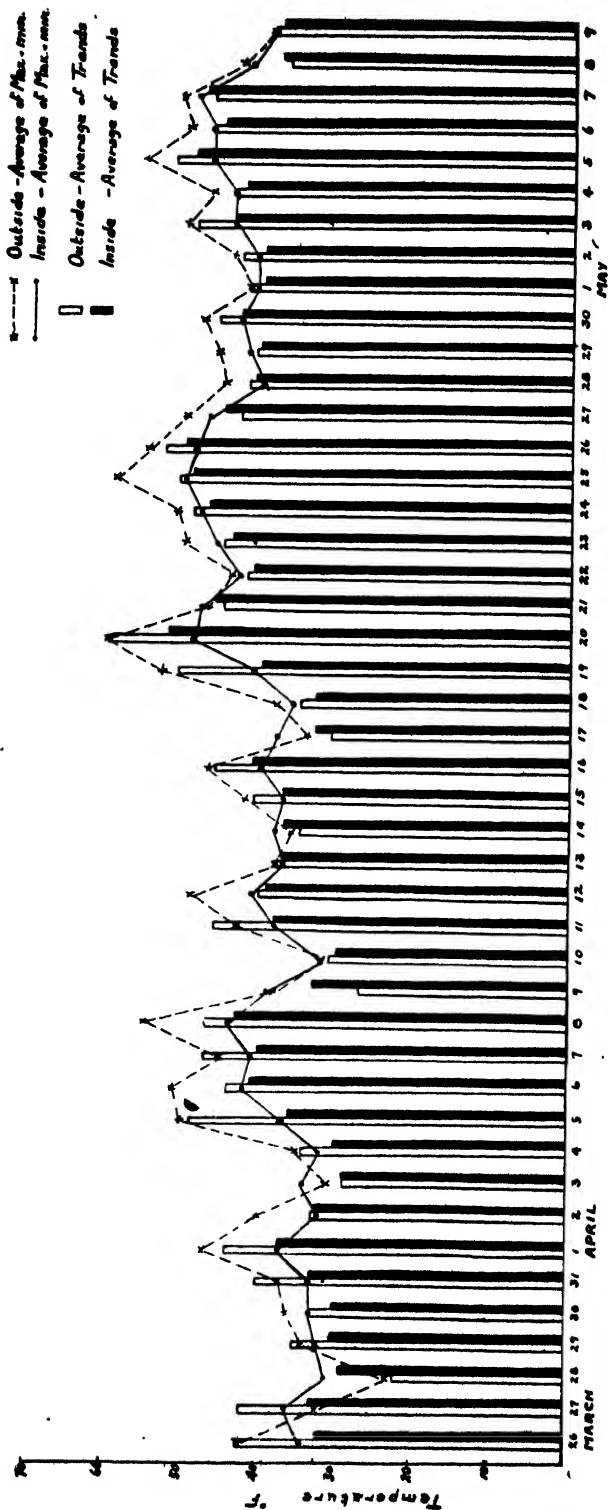


FIGURE 7. Daily averages of outside and inside temperatures as lines. Bars represent averages of temperature trends as shown on thermograph charts.

The season used for study began with the last week of adequately controlled storage temperature in the spring and ended when 32° F. was held steadily in the fall.

Tracings of thermograph charts (Figure 6) are typical of records obtained in the spring, summer, and fall seasons. Publication of all the charts would not only consume too much space but would not provide easily interpreted information.

Maximum-Minimum Temperature Averages

During the season selected for study, the temperatures both inside and outside the storages were fluctuating and it is therefore necessary to use averages to describe the conditions. One method used commonly is to average the maximum and minimum temperature for a given unit of time.

That method was adopted to study daily temperature relationships over a period of 45 days (Figure 7).

The daily average maximum-minimum outside is shown as a broken line and a solid line represents the corresponding averages inside the concrete storage. The average condition is approximately 6° F. higher temperature outside than inside.

A somewhat more accurate comparison of average temperatures is obtained by averaging the temperatures of the major temperature trends as seen on thermograph records. Such averages are shown in Figure 7 by vertical clear bars representing outside conditions and solid bars for inside temperatures. The average condition is approximately 3° F. higher temperature outside than inside with a range of daily difference up to 12° F.

It should be noted that the electric heater was disconnected prior to March 26 (when the records in Figure 7 began), but the cut-out thermostat was still operating to stop ventilation at 32° F. The storage was empty. Any drift below 32° F. was due to uncontrolled heat loss.

Inside temperatures were at or lower than 32° ± 1° F. during 13 of the 24 days to April 18. On all but 4 days the storage temperature was below 40° F.

After April 19 the storage temperature was ordinarily at or above 40° F.

Weekly Averages of Trends

Inside and outside temperature conditions for the season March 8 to December 6, 1948, are illustrated in Figure 8.

The broken line represents the maximum outside temperature for each week. The solid line represents the weekly average of outside daily average of maximum-minimum temperatures. Vertical bars represent average temperatures as determined by averaging the major trends on the thermograph charts. Clear bars, solid bars and cross-hatched bars illustrate, respectively, outside, concrete storage and frame storage temperatures.

Over the season in question the frame storage maintained a temperature averaging 6° F. lower (average trend temperatures) than the outside temperature as represented by averages of maximum-minimum temperatures.

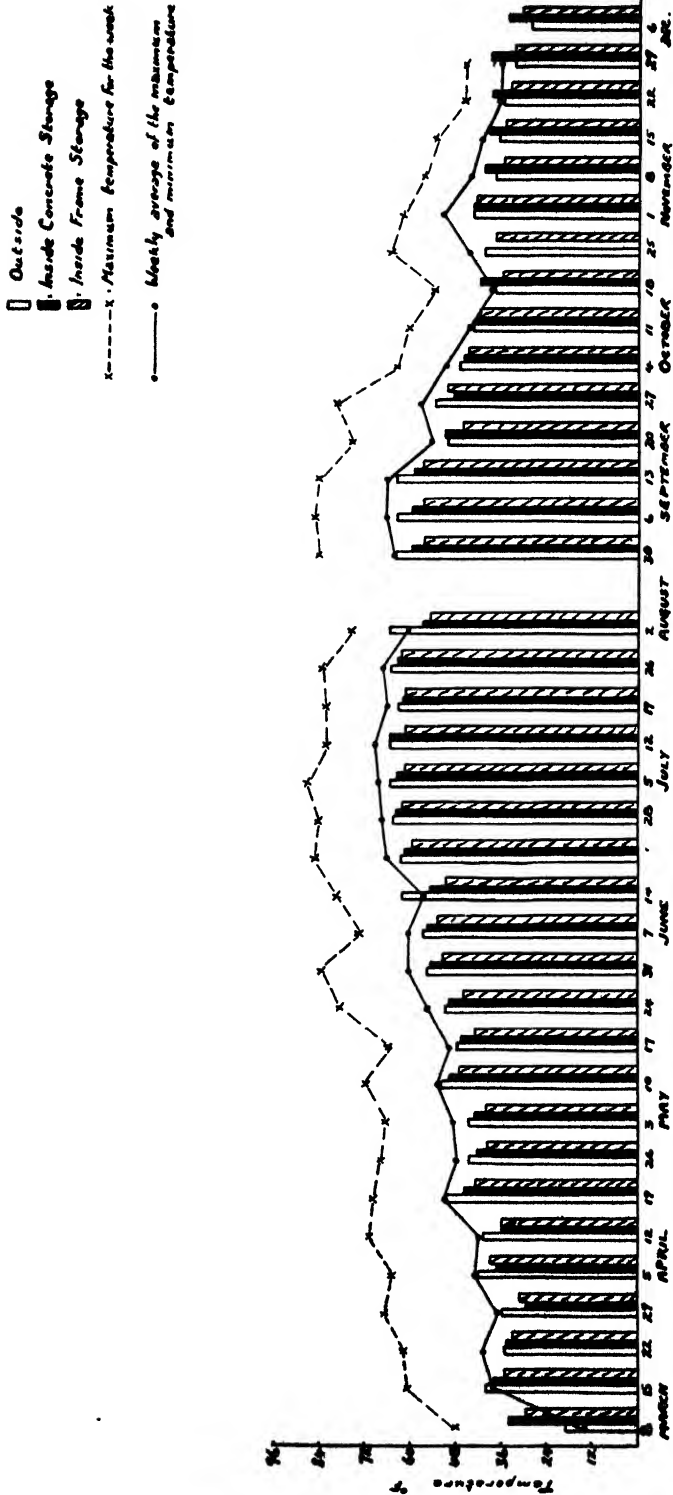


FIGURE 8. Weekly averages of outside daily maximum-minimum temperatures are shown as a solid line. A broken line represents maximum outside temperature for each week. Bars show averages of trends as seen in thermograph records.

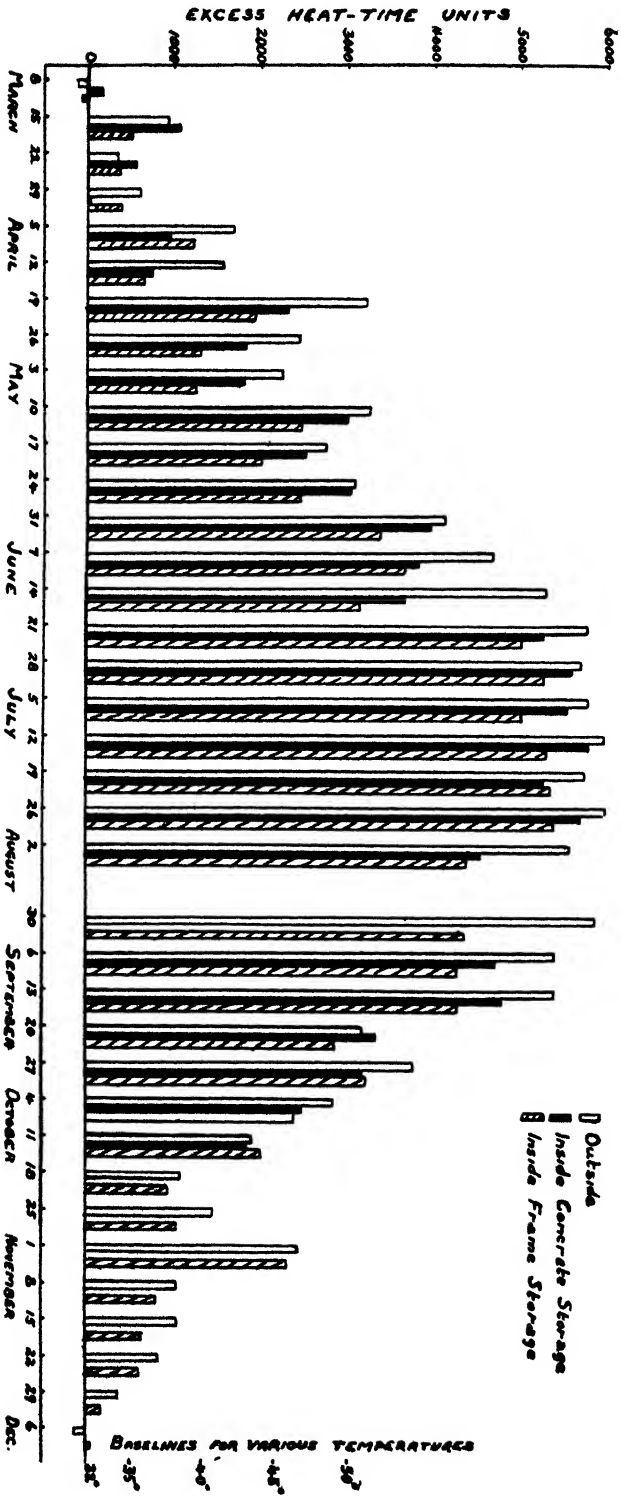


FIGURE 9. Heat-time unit comparisons of outside and inside conditions; 32° F was used as a base line.

Heat-Time Unit Comparisons

An accurate description of fluctuating temperatures takes into account both time and temperature. Each major temperature trend in the thermograph records was marked on each record. The average temperature of each trend was multiplied by the number of hours' duration of each trend. The sums obtained were totalled for each week to give a total of "heat-time" units for the week. If the storages were held constantly at 32° F. there would be only one storage trend with an average temperature of 32° F. maintained during the number of hours in a week. Subtraction of this sum from the heat-time units actually found provides a direct comparison of the relationship between inside and outside temperatures with duration of temperatures taken into account.

Such a comparison is made in Figure 9, where the weekly heat-time units in excess of those at 32° F. are shown as vertical bars. Clear, solid and cross-hatched bars represent respectively outside, concrete storage and frame storage conditions. Comparisons based on uniform temperatures higher than 32° F. can be made by drawing horizontal lines in the positions indicated on the right of Figure 9.

Appropriate horizontal lines show that the storages were above 50° F. from June 1 to October 4, above 40° F. from April 19 to October 18, and above 32° F. from March 8 to the end of November.

DISCUSSION AND SUMMARY

Prior to the development of a sensitive, automatic system of ventilation for common storages it was difficult to estimate possible performance, in terms of sustained temperatures, in this type of storage. The ventilation equipment described here operates primarily under control of a differential thermostat. The design of the thermostat is described in detail. The ventilation system appears to result in nearly optimum use of naturally refrigerated air.

Common storages are in very general use and there are some indications that their use may be expanded. It is believed that the performance of most of the existing storages may be greatly improved by adequate control of ventilation.

Since natural air varies in temperature it is necessary to describe the temperature in terms of averages per unit of time. Three methods of describing average conditions are presented. Daily maximum-minimum averages both inside and outside a storage are compared (Figure 7). Averaging the number of trends of temperatures shown in thermograph charts takes duration of temperature partially into account (Figures 7 and 8). Time-temperature units (Figure 9) emphasize duration of temperature. All three methods show that storage temperature averages lower than outside temperature.

Daily averages of maximum-minimum temperatures are obtainable from meteorological stations in most areas in Canada, whereas trend data may be more difficult to obtain and use. A comparison was made between the inside average of trend temperatures (on a weekly basis) and outside daily averages of maximum-minimum temperatures, averaged for each week, during March to December, 1948. The inside temperature averaged 6° F.

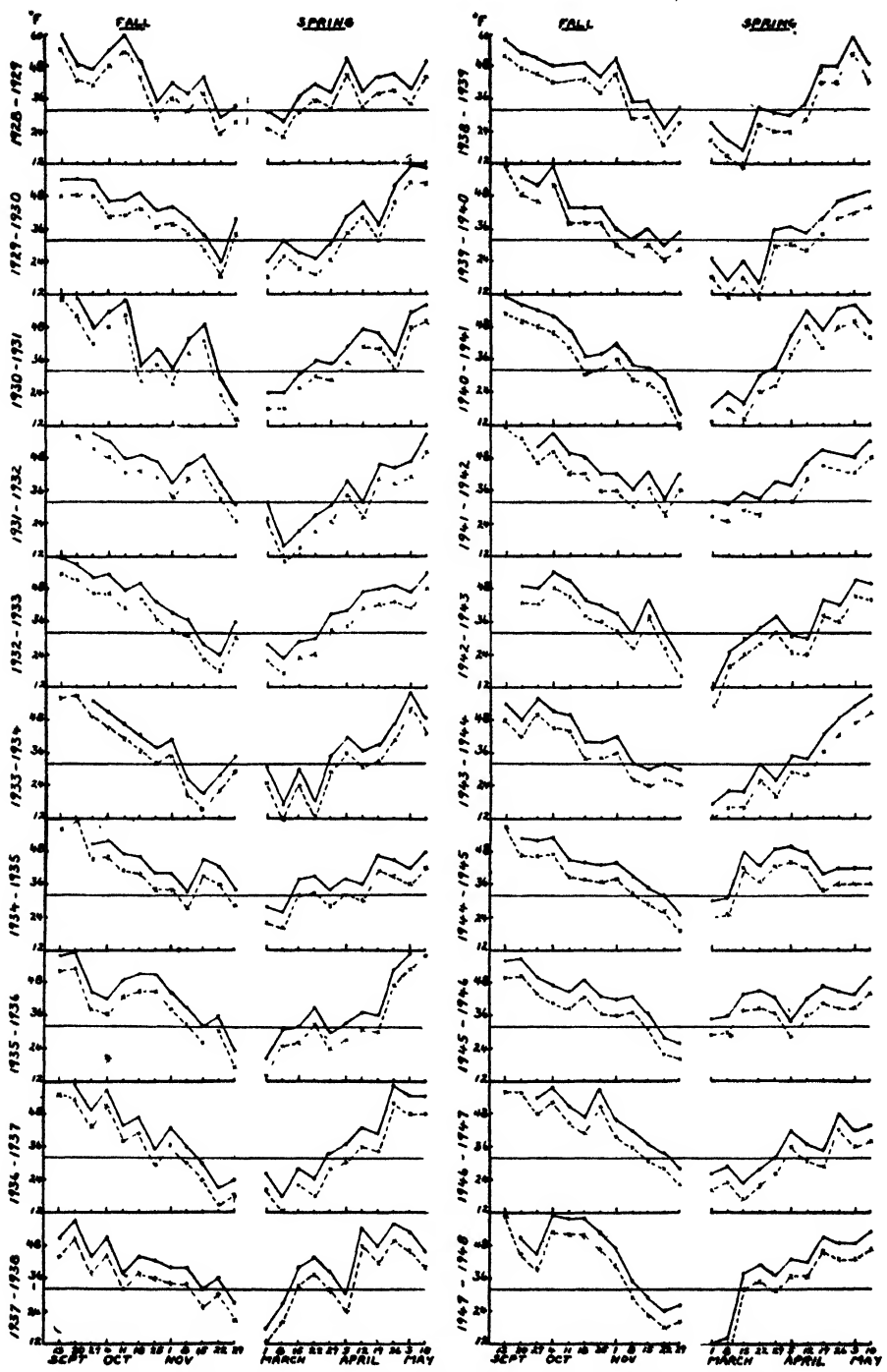


FIGURE 10. Outside weekly temperature averages for fall to spring storage terms, beginning 1928-29 and ending 1947-48. The broken line is the assumed storage temperature.

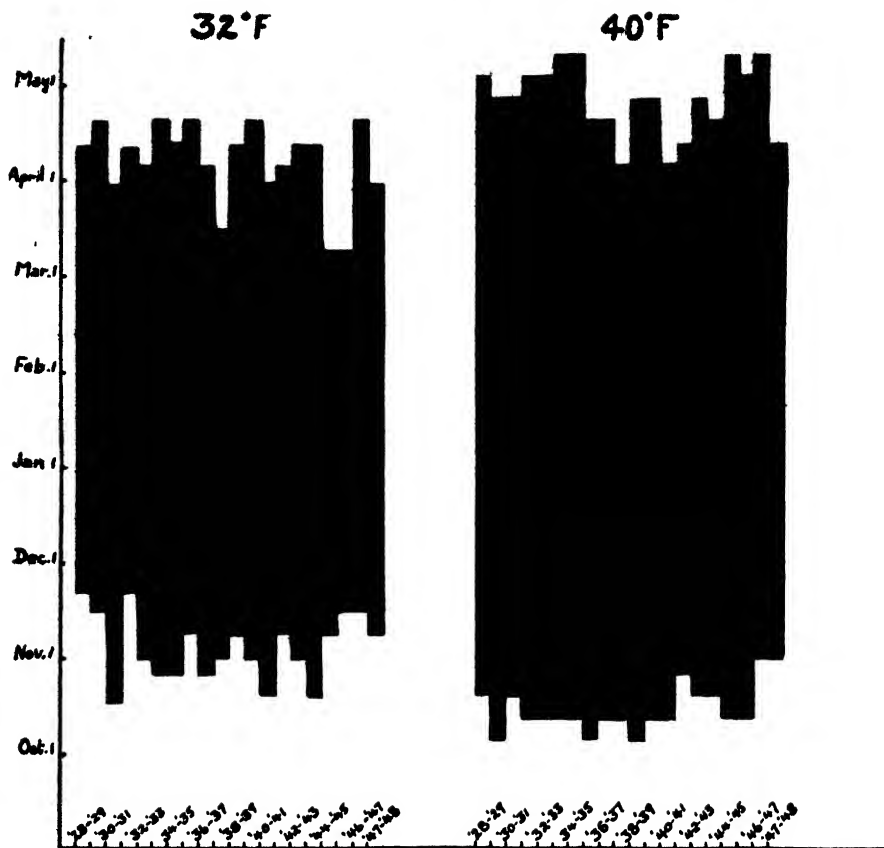


FIGURE 11. Charts showing estimated duration of storage temperatures of 32° F. and 40° F., respectively, over a 20-year period at Guelph.

below that outside during that period. It is now believed that an adequately insulated storage, with automatic ventilation, will perform, on the average, in such a manner that its temperature will be at least 6° F. below that represented by averages of outside maximum-minimum temperatures.

Outside average temperatures during the past 20 years here were plotted to show conditions during each fall and the following spring. The probable storage temperature was plotted 6° F. below outside averages (Figure 10). The probable duration, together with beginning and ending dates, for inside temperatures of 32° F. and 40° F. are charted in Figure 11. Averages of the 20-year, probable performance, are: 4.7 months at 32° F. with a range of 3.7 to 5.7 months; and 6.3 months at 40° F. with a range of 5.5 to 7 months.

Some estimates can be made of the possible performance of other adequately insulated above-ground storages.

Larger storages, if provided with relatively the same ventilation capacity, should perform similarly. Somewhat lower averages might be obtained in that the ratio of volume to exposure of walls and roof is more favourable in the larger storage.

The effect of loading in the fall is difficult to estimate in that the temperature of the incoming load will vary greatly and the method of packaging and stacking may limit the rate of cooling. However, if harvested materials are cooled overnight outside the storage it should be possible to obtain very little disturbance of the storage air temperature when the material is loaded in the morning. Load during winter and spring will serve to smooth inside temperatures and reduce the effectiveness of short periods of late spring low temperature air.

It is not possible to predict performance in storages subject to various sources of heat. Examples are storages at various depths underground, in heated cellars or in barns where one or more walls are subjected to barn temperatures.

The adoption of automatic controls for ventilation and of an auxiliary heat source (where necessary) has resulted in accurate winter control (approximately $\pm 1^\circ \text{F.}$) of temperature in an above-ground common storage. Estimates of performance during the past 20 years show that the principal weakness of the common storage is the variation in the date when 32°F. can be reached and maintained in the fall season. That date has varied between October 15 and December 1 during the past twenty years. The condition is, of course, ameliorated by the fact that temperatures somewhat above 32°F. have preservative effects. Early termination of the desired temperature in spring is less important in that much of the storage load has been consumed during the winter months.

The adoption of the automatic ventilation system has resulted here in much more efficient utilization of cool outside air than is a practical possibility with manual controls. Then since the thermostats are adjusted at factory according to the products stored, and since an adequate structure automatically adjusts itself to a temperature suited to the product, the grower is thereby presented with a convincing demonstration of the desirability of proper storage temperature, during the first year of operation. Reports to that effect have already been received from some of those who have used the system. Performance in the grower's storage seem to be a much more effective educational method than demonstrations in our experimental storages.

ACKNOWLEDGMENTS

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STUDY OF FERTILIZER UPTAKE USING RADIO-ACTIVE PHOSPHORUS

IV. THE AVAILABILITY OF PHOSPHATE CARRIERS IN CALCAREOUS SOILS

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In Western Canada, 11-48-0 ammonium phosphate is the usual phosphate fertilizer, and fertilizer trials have indicated that this form is superior to ammoniated superphosphate, 2-20-0, or triple superphosphate 0-38-0, the latter forms being calcium phosphates (9). Ensminger and Cope (4) have recently reported on a comparison of these materials in Alabama, and conclude that ammonium phosphate is an inferior source of phosphate under their conditions. Several such comparisons between different phosphate carriers have been conducted (2, 8) but relatively little precise information is available for the calcareous soils of semi-arid, sub-humid regions.

Preliminary results previously reported (3) had indicated large differences in availability between different forms of phosphate, suggesting that under soil conditions of neutral to alkaline reaction, calcium phosphates are relatively inferior sources of phosphate for wheat. In order to obtain more information on this point, and to check the tentative greenhouse conclusions in the field, the experiments reported here were set out in the 1948 season. Radioactive phosphorus was used as a tracer to determine accurately the amounts of fertilizer phosphorus taken up.

Comparisons made in the field (9), indicating that 11-48-0 ammonium phosphate is superior to 0-38-0, a triple superphosphate, have raised the question whether the superiority of 11-48-0 ammonium phosphate has been due entirely to the value of its nitrogen as a nutrient. Dressings of ammonium sulphate have given no general yield increases in field tests (12). The experiments reported here were designed to answer this question by comparing equal rates of phosphate application, the carriers being: (1) mono-ammonium phosphate; (2) mono-sodium phosphate; (3) mono-calcium phosphate; (4) dicalcium phosphate, and (5) dicalcium phosphate plus calcium nitrate, which gives a slightly higher nitrogen addition than that of the ammonium salt. In addition, two other rates of ammonium phosphate application were included, as well as an unfertilized treatment, to give additional information on the influence of rate of application on fertilizer uptake (2, 11, 17).

MATERIALS AND METHODS

Preparation of Fertilizers

The radioactive fertilizers were prepared by adding the radioactive phosphorus in the form of phosphoric acid to solutions from which the various phosphates were obtained by evaporation. In solution and in the crystallization process, the P^{32} tracer would distribute itself at random with regard to P^{31} , and hence would indicate exactly the fate of the phosphate in the fertilizer applied to the soil.

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Radioactive mono-ammonium and mono-sodium phosphates were prepared by evaporation at less than 45° C. of solutions of the respective salts, to which P^{32} as H_2PO_4 had been added. Crystal growth was controlled to some extent by the frequency of stirring during the evaporation.

The radioactive mono-calcium phosphate was prepared by dissolving mono-calcium phosphate and the radioactive phosphoric acid solution in water and recovering the material by evaporation as in the sodium and ammonium preparations. However, the lower solubility of the mono-calcium salt meant that very large quantities of solution would be required. This was avoided by incorporating the radioactivity, by solution and evaporation, into about one-quarter of the required amount of material, grinding to less than 100 mesh in an agate mortar, and mixing with similarly ground non-active material until tests for P^{32} on representative samples showed homogeneity within the limits of counting error.

The radioactive dicalcium phosphate was prepared by adding the P^{32} to the dicalcium phosphate dissolved in 25 per cent acetic acid. According to Larson (7), pure dicalcium phosphate is prepared by crystallization of about 5/6 of the dissolved material. To avoid the resulting delay in recovering the tracer lost, the preparations were evaporated to a thick paste, to recover as dicalcium phosphate all the tracer added. In this case also, the P^{32} was incorporated into only a fraction of the desired quantity, the required amount being obtained by grinding and mixing until homogeneity was achieved, within the limits of error of the counting system.

The radioactive mixture of dicalcium phosphate and calcium nitrate was prepared by mixing the ground active dicalcium phosphate with ground calcium nitrate.

The phosphates, in a semi-liquid crystalline state, were pelleted by forcing the moist mass through a sieve of suitable mesh, allowing it to dry, sieving out the desired size fraction, and repelleting the finer fractions. This was done to simulate as closely as possible the coarse granular condition of the commercial products used on the Canadian prairies.

Precautions were taken to avoid radiation hazards from the P^{32} and to avoid inhalation of radioactive dust.

Plot Arrangement

To give some factor of insurance against weather and other hazards, identical trials were laid down at Aberdeen on Elstow Clay, a chestnut soil on shallow glacial lacustrine material; at Humboldt on Naicam Loam, a chernozem on resorted glacial till, and at Birch Hills on Melfort Silty Clay Loam, a thick chernozem on deep glacial lacustrine deposits (10).

The design of the trials was a modified Latin square of 4 replicates and 4 harvest dates, to permit following fertilizer uptake throughout the growing season to harvest. Each block consisted of 8 treatments, as shown in Table 1, with full randomization within each block.

Each treatment plot consisted of 5 rows 9 feet long, 6 inches apart. The two outside rows were guard rows, and were fertilized uniformly with 11-48-0 commercial ammonium phosphate at a rate of phosphate equivalent to that of the treatment. The three inner rows were given the designated

fertilizer treatment, with only the middle row being fertilized with radioactive fertilizer. This arrangement was adopted so that analysis of the two rows adjacent to the tracer row would indicate how much of the fertilizer applied to the middle row was being taken up by the plants of the adjacent rows, and conversely, how much of the P^{31} in the plants of the tracer row came from the fertilizer of adjacent rows. This potential source of error was controlled in this manner, and further, the method permitted an estimate of the degree to which one row of plants feeds on fertilizer in the root zone of another. A comparison of plant weight from the tracer rows and adjacent rows with the same fertilizer treatment also permitted an estimation of any effects of P^{32} itself on the growth of the above-ground parts, to give further information on the dangers of injury reported by Scott-Russell and Martin (13). The centre 6 feet of the middle row was harvested for yield and for analysis. The four harvest dates were chosen to give plants approximately at the following stages of development:

- | | |
|---------------------|-----------------------|
| I—shot-blade stage, | III—soft-dough stage, |
| II—heading, | IV—maturity. |

The treatments, together with the amounts of nutrients supplied, and the concentration of P^{32} , are given in Table 1 below. A higher initial concentration of P^{32} was necessary for those blocks going to maturity to allow for the decay factor.

TABLE 1.—FERTILIZER TREATMENTS

Material	Nutrients supplied, lb./acre		Activity of tracer fert. $\mu\text{c/gP}^{31}$ *	
	P_2O_5	N.	First 3 harvests	Fourth harvest
1. Mono-ammonium phosphate; half rate	12	2 8	•	
2. Mono-ammonium phosphate; basic rate	24	5 5		
3. Mono-ammonium phosphate; double rate	48	11	35 μc	360 μc
4. Mono-sodium phosphate	24	0		
5. Mono-calcium phosphate	24	0		
6. Dicalcium phosphate	24	0		
7. Dicalcium phosphate—calcium nitrate	24	6 4		
8. Unfertilized	0	0		

* As at May 12, 1948.

The plots were seeded late in May, 1948, with Thatcher wheat at $1\frac{1}{2}$ bushels per acre, by means of a Kemp V-belt rod-row seeder, the grain and fertilizer being sown together. The metal wind deflector on the seeder was replaced by one of plate glass, to give protection against radiation and to give visibility, while the seed and fertilizer were being distributed on the V-belt.

Analytical Methods

The plant material from the harvested 6-foot portion of the row was dried at 105°C . and weighed. For analysis, the material was finely ground and mixed in a mechanical shaker. Aliquots of suitable size for

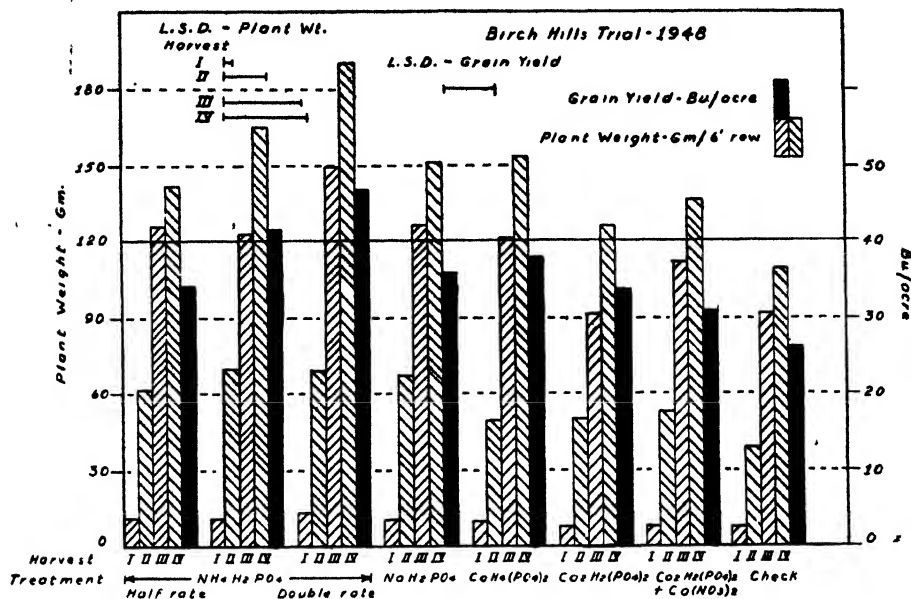


FIGURE 1

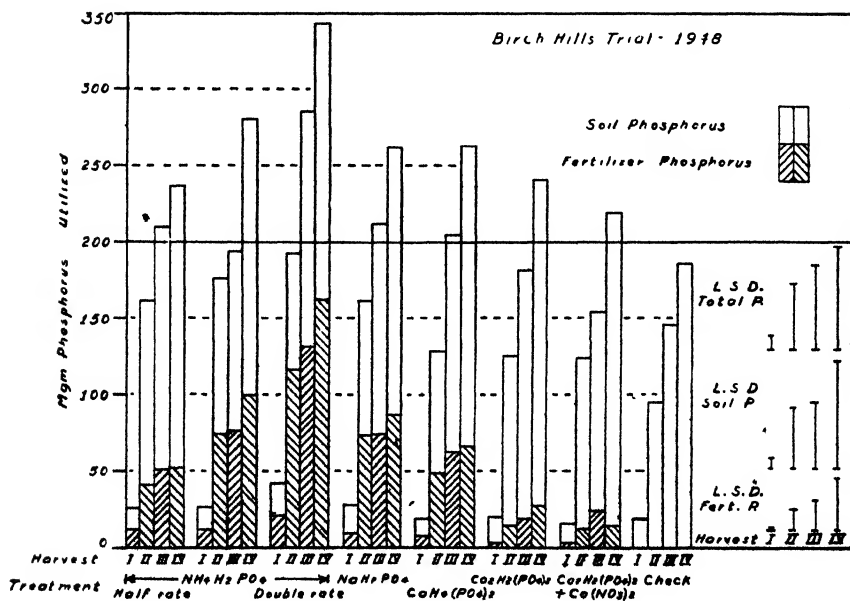


FIGURE 2

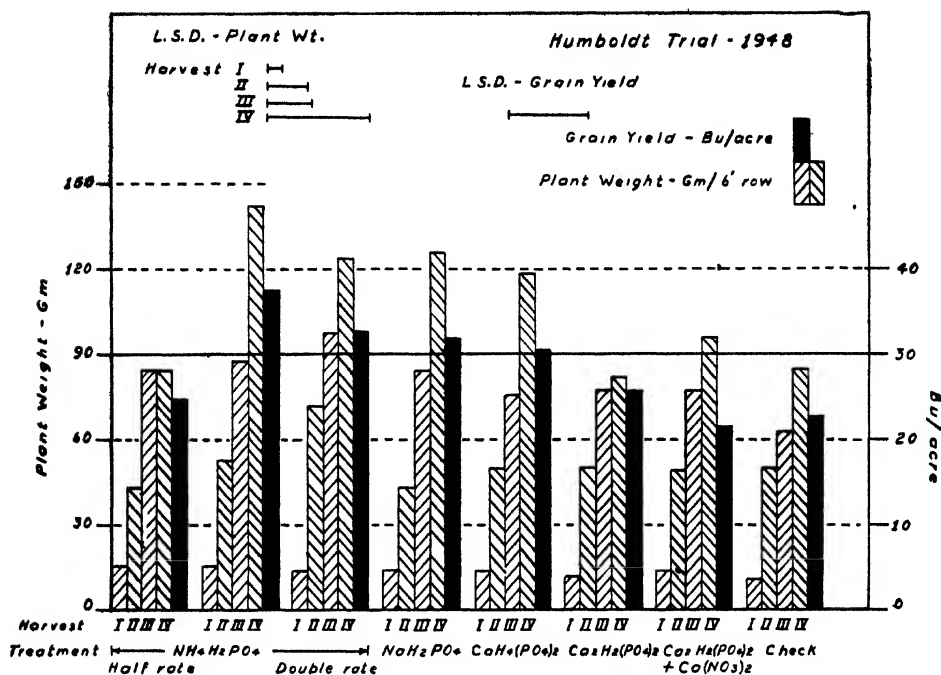


FIGURE 3

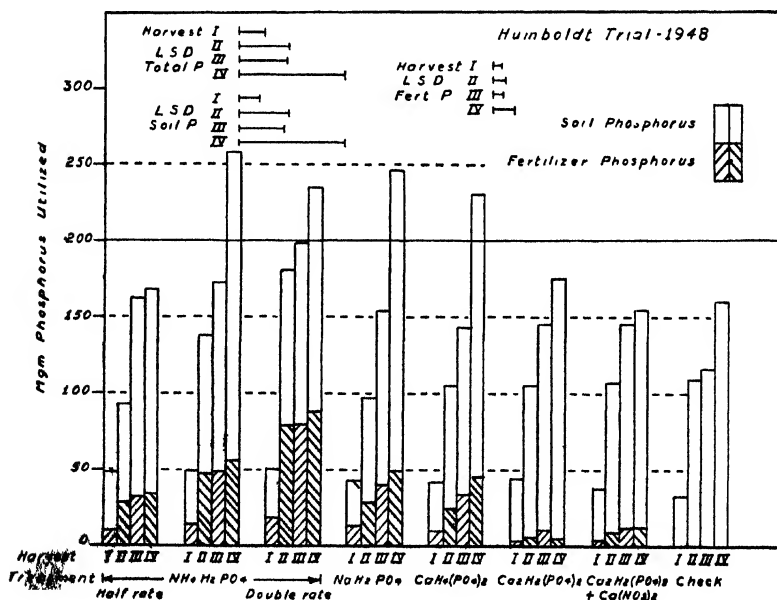


FIGURE 4

phosphorus analysis were wet ashed by the method of Brenner and Harris (1). After diluting to a known volume and filtering, portions of the solution were used for the estimation of total phosphorus by the colorimetric method suggested by Shelton and Harper (14), and for the estimation of P^{32} , according to previously described methods, involving the correction for self-absorption (15).

The sample taken at maturity was hand-threshed to obtain the weight of grain and straw. The grain and straw were then ground separately, and the phosphorus determinations carried out on a sample containing grain and straw material in the same proportion as determined at threshing.

RESULTS

Some difficulty was experienced with germination, due to the very hot, dry spring, and at one location, Aberdeen, early growth was so slow and irregular that the trial was discarded, while at Birch Hills one block germinated so poorly as a result of a seeding accident that it was treated as a series of missing plots for the harvest affected.

In general, growing conditions were not good during the early part of the year due to high temperatures and lack of rain. The normal June rains appeared in July, however, and yields were good, although the season was late.

Large differences could be observed in the growth of the various plots, ammonium phosphate being outstanding. The dicalcium phosphate preparations were relatively ineffective, on a basis of visual estimates.

The above-ground portions were taken for determination of oven-dry weight and phosphorus and radiophosphorus content. The results are given in Table 2 (a), 2 (b), and Table 3. The numerical data of Table 2 are presented in simpler form in Figures 1, 2, 3, and 4, as histograms. The required differences for significance are included in Figures 1 to 4, using standard methods of statistical analysis (5).

Various observations may be made on different aspects of the experiment.

Rate of Application

It is obvious that as the rate of application of ammonium phosphate was increased, the plants took up additional amounts of fertilizer phosphorus, but the proportion of the applied phosphate taken up decreased. The values in the last column of Table 2 (a) and (b) indicate a steady decrease in the percentage of the applied phosphate utilized by the plant, in comparing the half, standard and double rate of application of $NH_4H_2PO_4$. It is interesting to note that in the case of the double rate of $NH_4H_2PO_4$, the fertilizer phosphorus supplied very nearly half of the plant's total requirement for phosphate.

Availability of Different Carriers

In comparing the availability of the different carriers, the data show some divergence between locations. In all cases, the dicalcium phosphate and dicalcium phosphate plus calcium nitrate were only slightly available, with the nitrate-phosphate preparation being consistently superior but not

TABLE 2 (a).—YIELD AND ANALYTICAL DATA, BIRCH HILLS, 1948

(Sown, May 26, 1948)

Averages of four replicates

	Mono-ammonium phosphate			Mono-sodium phosphate	Mono-calcium phosphate	Dicalcium phosphate	Dicalcium phosphate + $\text{Ca}(\text{NO}_3)_2$	Un-fertilized	L.S.D. 5 per cent. pt.
	Half rate	Basic rate	Double rate						
Plant weight gm./6-ft. row									
I June 21	10.9*	10.8*	13.4*	10.6*	9.5*	7.6*	8.1*	7.6*	2.9
II July 7	62.4	70.2	69.4	67.3	49.6	50.3	52.8	38.6	17.5
III July 23	126.2	122.7	149.0	125.9	120.7	91.0	112.1	91.4	31.7
IV Aug. 24	142.9	165.0	189.9	150.5	153.4	125.8	136.2	108.5	34.5
Grain yield—bu./acre	34.1	41.6	46.8	35.9	37.8	30.9	33.8	26.1	8.1
Total P., mg./6-ft. row									
I June 21	25.8*	27.0*	42.7*	27.7*	18.9*	16.7*	19.7*	18.8*	8.8
II July 7	162.3	177.2	194.0	161.5	128.9	124.2	126.1	95.0	44.3
III July 23	211.2	195.1	285.9	211.8	205.4	154.8	181.6	145.9	56.3
IV Aug. 24	236.3	280.7	343.4	261.9	262.3	219.5	240.7	188.3	67.7
Fert. P. taken up mg./6-ft. row									
I June 21	12.2*	12.5*	21.3*	10.1*	7.1*	2.7*	1.9*	—	1.4
II July 7	41.4	75.5	117.2	74.4	49.3	12.6	15.1	—	13.6
III July 23	52.1	77.3	132.4	74.5	62.9	19.1	19.1	—	20.2
IV Aug. 24	52.8	100.7	163.6	87.5	66.4	13.8	21.9	—	34.3
Soil P. taken up mg./6-ft. row									
I June 21	13.8*	14.5*	21.4*	17.6*	11.8*	14.0*	17.8*	18.8*	7.1
II July 7	120.9	101.7	76.8	88.0	79.7	111.6	111.0	95.0	39.9
III July 23	159.0	117.3	153.5	139.3	142.5	135.8	160.3	145.9	43.3
IV Aug. 24	183.5	180.1	179.8	174.4	195.9	205.8	218.7	188.3	70.3
Fert. P. taken up as per cent of Fert. P. applied									
I June 21	7.5	3.8*	3.3*	4.1*	2.2*	2.8*	0.6*	—	—
II July 7	23.5	23.0	17.9	22.4	15.0	3.9	4.6	—	—
III July 23	31.7	23.6	20.2	22.7	19.2	5.8	5.8	—	—
IV Aug. 24	32.2	30.7	25.0	26.7	20.3	4.2	6.7	—	—

* One replicate calculated as a missing plot.

TABLE 2 (b).—YIELD AND ANALYTICAL DATA, HUMBOLDT, 1948

(Sown, May 19, 1948)

Averages of four replicates

	Mono-ammonium phosphate			Mono-sodium phosphate	Mono-calcium phosphate	Dicalcium phosphate	Dicalcium phosphate + Ca(NO ₃) ₂	Un-fertilized	L.S.D. 5 per cent pt.
	Half rate	Basic rate	Double rate						
Plant weight, gm./6-ft. row	15.0	15.6	13.7	14.1	13.7	11.8	13.6	10.4	5.9
I June 21	43.1	53.5	72.1	42.9	50.8	49.4	49.1	49.7	16.0
II July 8	84.7	87.7	97.5	84.0	75.3	77.7	77.5	62.9	18.5
III July 23	84.4	141.6	123.2	125.7	117.8	81.5	95.9	84.6*	40.7
IV Aug. 19									
Grain yield—bu./acre	24.8	37.7	32.7	31.9	30.5	25.8	21.3	22.7*	10.5
Total P., mg./6-ft. row	48.9	49.7	50.9	43.5	42.5	38.6	44.7	33.3	19.0
I June 21	92.9	138.5	181.7	97.3	105.9	107.4	106.1	109.7	37.0
II July 8	163.1	172.8	199.7	154.2	143.5	146.3	146.0	116.5	35.6
III July 23	168.9	258.5	236.9	247.2	230.7	155.5	174.4	160.7*	78.0
IV Aug. 19									
Fert. P. taken up mg./6-ft. row	10.6	14.6	19.4	12.3	10.1	2.6	3.7	—	6.3
I June 21	28.1	47.5	80.4	28.2	24.6	4.9	7.8	—	9.0
II July 8	32.2	48.8	80.7	40.7	34.1	10.1	10.7	—	7.7
III July 23	34.7	56.8	88.8	49.5	46.3	4.8	11.7	—	15.9
IV Aug. 19									
Soil P. taken up mg./6-ft. row	38.3	35.1	31.5	31.2	32.4	35.9	41.0	33.3	15.8
I June 21	64.8	91.2	101.3	69.1	81.3	102.6	98.3	109.7	37.0
II July 8	130.9	123.9	119.0	113.5	109.5	136.1	135.3	116.5	33.5
III July 23	134.1	201.7	148.2	197.7	184.3	150.8	162.7	160.7*	78.5
IV Aug. 19									
Fert. P. taken up as per cent of Fert. P. applied	6.5	4.5	3.0	3.8	3.1	0.8	1.1	—	—
I June 21	17.1	14.4	12.3	8.6	7.5	1.8	2.4	—	—
II July 8	19.7	14.9	12.4	12.4	10.4	3.1	3.3	—	—
III July 23	21.2	17.4	13.6	15.1	14.2	1.5	3.6	—	—
IV Aug. 19									

* Figures for two plots calculated as missing plots.

significantly so. The more soluble materials consistently rank themselves (from most available to least) as ammonium, sodium, and mono-calcium phosphate. For the later stages of growth, there are no significant differences between ammonium and sodium phosphates, and no significant differences between sodium and mono-calcium phosphates at any one location. At both locations, mono-calcium phosphate is significantly less available than ammonium phosphate, and significantly more available than either dicalcium phosphate or the dicalcium phosphate-calcium nitrate material.

A comparison of average fertilizer uptake in Table 3 at the two locations for the last harvest for the various carriers indicates the same situation as in the individual cases, with ammonium phosphate being significantly better than either sodium phosphate or mono-calcium, but the differences between sodium and mono-calcium phosphate being too small to be statistically significant on this test. The dicalcium phosphate preparations are markedly less available than the more soluble forms.

The nitrogen content of the material does not greatly change the amount of phosphate the plant recovers, although the indications are that the $\text{Ca}_2\text{H}_2(\text{PO}_4)_2 - \text{Ca}(\text{NO}_3)_2$ preparation is slightly better than $\text{Ca}_2\text{H}_2(\text{PO}_4)_2$, and that $\text{NH}_4\text{H}_2\text{PO}_4$ is slightly better than NaH_2PO_4 , suggesting a small beneficial effect from nitrogen on phosphate uptake from fertilizers. However, comparing these four materials on the basis of presence or absence of nitrogen gives no statistically significant effect of nitrogen on phosphate uptake under the conditions of this experiment.

The relative values of the carriers tested here are quite different from those obtained by Ensminger and Cope (4). The different behaviour of the various materials in Alabama and in Saskatchewan is not surprising, since soil conditions are diametrically opposite, being humid and acid, and semi-arid and alkaline, respectively.

In semi-arid soils, reactions of pH 7.0 and above are the rule, with plentiful supplies of calcium. Under these circumstances, precipitation of phosphate as $\text{Ca}_3(\text{PO}_4)_2$, or precipitation and adsorption of phosphate ions on the surface of calcium carbonate particles is to be expected. The extreme importance of calcium in these reactions makes it appear that the most effective fertilizer would be one with a small tendency to conversion to $\text{Ca}_3(\text{PO}_4)_2$, and consequently one with no calcium, and that effectiveness could be expected to decrease as the calcium content of the material increases. In general, then, the greater the difference of the material from tri-calcium phosphate, the greater is its effectiveness, and conversely, the more nearly its form approaches tri-calcium phosphate, the faster will it be changed to difficultly available tri-calcium phosphate, and therefore the lower will be its effectiveness.

This demonstration of a big difference in availability of phosphate in various carriers has broad implications. We can say that the superiority of ammonium phosphate over calcium phosphates, as a fertilizer under neutral to alkaline soil conditions, is primarily because it remains available longer rather than because of the marked nutritional benefits of the nitrogen contained in the ammonium phosphate. On the brown and chestnut soils, where there is no general nitrogen deficiency that can be corrected with

TABLE 3.—SUMMARY OF DATA AT MATURITY, HUMBOLDT AND BIRCH HILLS COMBINED, 1948

	Mono-ammonium phosphate			Mono-sodium phosphate	Mono-calcium phosphate	Dicalcium phosphate	Dicalcium phosphate + Ca(NO ₃) ₂	Un-fertilized	L.S.D. 5 per cent pt.
	Half rate	Basic rate	Double rate						
Fert. P. mg./6-ft. row	43.7	78.8	126.1	68.5	56.4	9.2	16.8	—	17.8
Soil P. mg./6-ft. row	158.8	190.8	164.0	186.1	190.1	178.3	190.7	174.5	51.8
Total P. mg./6-ft. row	202.3	269.6	290.1	254.5	246.5	187.5	207.5	174.5	50.1
Plant weight, gm./6-ft. row	113.7	153.3	156.6	138.1	135.6	103.6	116.0	96.5	26.6
Grain yield, bu./acre	30.5	41.2	41.3	35.2	35.4	27.1	30.8	25.3	7.25

TABLE 4.—FERTILIZER TAKEN UP FROM THE ACTIVE ROWS BY ADJACENT ROWS
(Expressed as percentage of applied phosphate)

Location and harvest	Ammonium phosphate, half rate	Ammonium phosphate, basic rate	Ammonium phosphate, double rate	Sodium phosphate	Mono-calcium phosphate	Dicalcium phosphate	Dicalcium phosphate + Ca(NO ₃) ₂
Birch Hills I June 21	0.08	<0.01	<0.04	<0.01	<0.03	<0.01	<0.01
II July 7		0.15					
III July 23		0.57					
IV Aug. 24		0.80					
Humboldt I June 21	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
II July 8		0.09					
III July 23		0.28					
IV Aug. 19		0.40					

ammonium sulphate (12), it would seem that mono-ammonium phosphate or other soluble phosphates containing little or no calcium are very suitable carriers. Until general nitrogen deficiencies on brown and dark brown soils occur, an increase in the proportion of nitrogen to phosphorus in fertilizers of the ammonium phosphate type will be of no value, since the choice of ammonium phosphate as the best material available as a commercial fertilizer is based on the fact that it is not a calcium phosphate. In more humid regions, where nitrogen deficiencies may be a problem, the situation may be quite different.

A further implication lies in the fact that mono-ammonium phosphate and the calcium phosphates as in superphosphate differ in their availability as well as in their nitrogen content. Therefore, a comparison of fertilizer treatments, involving the use of a standard application of phosphate of different forms with varying amounts of nitrogen, is valueless for measuring nitrogen response. The nitrogen response will be complicated by a varying phosphate effectiveness, and only when the phosphate dressing is made with one uniform form of phosphate will a valid nitrogen response be measured. Many fertilizer tests will have to be reviewed from this aspect.

PLANT WEIGHT AND PHOSPHORUS CONTENT

From Figures 1, 2, 3 and 4, it can be seen that there is a close correlation between total phosphorus content and yield of plant material, under the conditions of this experiment. This suggests that plant growth is limited by phosphorus, or that there is no "luxury" consumption of phosphate. It can also be demonstrated from the data that the phosphorus content of the plants in the early stages is higher than at later stages, possibly indicating higher requirements for actively growing tissue. We may conclude that the beneficial action resulting from the addition of phosphate fertilizer is largely due to the fact that the fertilizer phosphate enables the plant to make a more vigorous early start, the later growth being completed largely from phosphorus absorbed from the soil.

Soil Phosphorus

An earlier paper from this laboratory (16) indicated that the fertilized crop took up appreciably more phosphorus from the soil than the unfertilized. The experiment reported here is the most recent one of a series in which this point has been carefully examined. In no case have we been able to verify statistically the earlier observation, and it is suggested that perhaps the earlier result was aberrant, and by no means the general rule. In this experiment, there were no significant differences between the average uptake of soil phosphorus at maturity for fertilized and unfertilized treatments. However, at earlier harvest dates, there were statistically significant differences between the amount of soil phosphorus taken up by the crop under different treatments, suggesting that comparisons of fertilizer uptake by subtracting the amount of nutrient taken up by a control plot may be in error. One difficulty in evaluating the importance of the variability in soil phosphorus uptake is the large standard error obtained in the statistical analysis, indicating large uncontrolled variations which may be masking real differences.

Feeding from Adjacent Rows

The data indicating the amount of tracer phosphorus, and hence fertilizer phosphorus, taken up by rows adjacent to the active rows, are given in Table 4.

The rows fertilized with inactive phosphorus adjacent to the row fertilized with radioactive phosphorus did not take up significant amounts of the tracer, and it is concluded that the root systems of two adjacent rows, 6 inches apart, are not intermingled in the vicinity of the original seed. This does not imply that wheat plants will not feed on fertilizer 6 inches away horizontally from the original seed, but does suggest that at the relatively shallow depth considered here—2 in.-3 in.—the root systems of adjacent rows do not compete for fertilizer sown with the seed. The fertilizer sown in one row is effective in that row only, with only very minor exceptions. It is also evident that in these particular experiments, no serious errors would be incurred by neglecting the loss due to fertilizer phosphorus taken up by adjacent rows.

Effect of Radioactivity

Recently, Scott-Russell and Martin (13) re-examined the possibility of injury to plants from radioactive materials used as tracers. They arrived at the conclusion that it is very possible to use radioactive isotopes in tracer experiments at levels harmful to plants, and therefore to give spurious results. Hendricks and Dean (6) had earlier explored this possibility and concluded that for P^{32} , there was a very comfortable margin of safety.

In estimating the amount of "active" fertilizer taken up by inactive rows, the above-ground parts of numerous plots have been harvested and weighed, permitting a direct comparison of the yield from active and inactive rows, all other factors being similar. These data are presented in Table 5.

At the first harvest date, some irregularity in germination had a slight influence on the results. Since the "active" row was of primary interest, the 6 feet of "active" row were chosen from the 9 feet available to give the most uniform section. It very occasionally happened that, in this way, sections of adjacent rows that had poor germination were taken. This may account for the higher values in the case of the "active" rows for the first harvest. The statistical analysis was according to Student's Pairing Method (5), using the values for the individual plots, by comparing the average yield of the two adjacent "inactive" rows with the yield of the "active" row.

The most likely treatments to suffer any possible injury from radiation in this experiment were those treated with the double rate of mono-ammonium phosphate. The absolute uptake of fertilizer phosphorus by the plants was highest for this treatment, and consequently, the tissues would have a higher concentration of the radioactive isotope in this treatment. The mean data for this treatment for all harvests at both locations are presented in Table 5 (b).

Examination of the data indicates no suggestion of any injury from the radioactive phosphorus, in comparing "active" and "inactive" rows, based on the weight of the above-ground parts. Since this comparison shows no

TABLE 5.—EFFECT OF RADIOACTIVE PHOSPHORUS ON PLANT GROWTH

(a) First harvest, all treatments recorded

	Plant wt., gm./6-foot row			
	Birch Hills		Humboldt	
	Active rows	Inactive rows	Active rows	Inactive rows
Ammonium phosphate—half rate	10.9*	10.0*	13.9	10.2
Ammonium phosphate—basic rate	10.7*	10.8*	16.1	14.6
Ammonium phosphate—double rate	12.3*	11.0*	13.7	8.2
Sodium phosphate	10.5*	10.6*	14.4	11.6
Mono-calcium phosphate	9.6*	9.8*	14.1	12.4
Dicalcium phosphate	7.6*	6.9*	10.7	11.3
Dicalcium phosphate + $\text{Ca}(\text{NO}_3)_2$	8.3*	7.9*	12.9	10.0
Mean	10.0	9.6*	13.7	11.6
L.S.D.—5 per cent pt.	0.8		1.9	

Mean of all treatments, both locations:

Active rows 11.8

Inactive rows 10.4

L.S.D. 5 per cent pt. 1.0

(b) All harvests—ammonium phosphate, double rate only

	Plant wt. gm./6-foot row		L.S.D. 5 per cent pt.
	Active rows	Inactive rows	
Birch Hills I	12.3*	11.0*	
II	69.4	72.5	
III	143.4	139.2	
IV	189.9	189.2	
Mean of 4	103.7	103.0	12.0
Humboldt I	13.7*	8.2*	
II	72.1	71.2	
III	97.4	86.3	
IV	123.2	124.6	
Mean of 4	76.6	72.6	8.8
Mean of all locations, all dates	90.2	87.8	9.6

All figures the mean of 4 plots except those marked *, indicating mean of 3 plots.

differences even at maturity, we can safely conclude that no significant effects on growth resulted from the presence of the radioactive phosphorus in the tissues of the plant.

The data confirm the earlier conclusion that it is relatively easy to avoid plant injury using radioactive phosphorus in tracer amounts in field experiments.

SUMMARY

An experiment is reported on the effects of rates and forms of phosphate on plant uptake of phosphorus on wheat under semi-arid conditions, with neutral to alkaline soils containing high levels of calcium. From the data, it is concluded that:

1. Increasing the rate of application of mono-ammonium phosphate gives greater fertilizer phosphate uptake, but decreases the percentage of fertilizer utilized.

2. In comparing various carriers of phosphate at the same rate of applied phosphorus, the carriers arranged themselves in order of effectiveness as follows:

- i. Mono-ammonium phosphate,
- ii. Mono-sodium phosphate,
- iii. Mono-calcium phosphate,
- iv. Dicalcium phosphate plus calcium nitrate,
- v. Dicalcium phosphate.

The dicalcium form was relatively ineffective, while mono-calcium phosphate was moderately available. The most effective forms were those without calcium, the mono-ammonium and mono-sodium salts, indicating that lower availability is probably related to the ease and rapidity of conversion to the difficultly available tri-calcium phosphate. In comparing similar phosphate carriers with and without nitrogen, the importance of the nitrogen on the effectiveness of the fertilizer was apparently small under the conditions of the experiment.

3. The total phosphorus taken up by the plant shows a good correlation with amount of growth, with evidence that the younger plants have a higher relative phosphorus content than plants at later stages of growth. However, any "storage" of phosphorus in the early stages does not account for the increased growth of the mature plant, but rather, it seems likely that since growth and phosphorus uptake are more or less parallel, increased uptake in the early stages means a higher final uptake, with a correspondingly greater growth.

4. One row of plants does not feed appreciably on fertilizer applied with the seed in adjacent rows 6 inches apart under the conditions of this experiment.

5. The effect on plant growth of radiation, and of radioactive phosphorus taken into the plant, is negligible. There is no evidence of injury from tracer phosphorus used in reasonable amounts in the field.

ACKNOWLEDGMENTS

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EXPERIMENTS IN THE CONTROL OF *SIMULIUM ARCTICUM* MALLOCH BY MEANS OF DDT IN THE SASKATCHEWAN RIVER¹

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INTRODUCTION

The river-breeding black fly, *Simulium arcticum* Mall., has been responsible in recent years for heavy losses of live stock throughout an area of approximately 10,000 square miles in the north-central agricultural area of Saskatchewan (4). These losses always occur in late May or early June as the result of the sudden emergence and mass flights of the flies. Protection of live stock from these flights has been difficult because it is almost impossible to predict the time and place at which an attack will occur. Additional flights, which occur in midsummer, have never caused serious losses of live stock.

The immature life-stages of the flies which form these outbreaks develop on scattered, extensive, rocky areas in the swiftly flowing waters of the relatively large North and South Saskatchewan Rivers. These breeding areas (Figure 1) occur at varying intervals throughout a distance of over 40 miles in the North branch between Prince Albert and the confluence with the South branch, and for a distance of over 175 miles in the South branch from Saskatoon to the confluence. The most important of these are at Fish Creek, Fenton and Prince Albert.

Results of investigations over a period of years have shown that the only certain method of preventing outbreaks is to eliminate the larvae of the first generation. The period when larvae only are present in the streams is short, about three weeks, and usually comes in the middle of May, but may be delayed into the latter part of May.

The field tests described in the paper were designed primarily to determine the practicability of treating the Saskatchewan River with DDT as a means of preventing the destructive spring fly flight of *S. arcticum*.

In 1948 the level of the rivers was considerably above normal, owing to exceptionally heavy precipitation in winter and early spring. As a result, many rocky beds, commonly inhabited by black fly larvae, were covered to a depth that made examination difficult. The marked increase in siltiness of these normally muddy rivers also contributed to the difficulties inherent in these investigations. However, accessible infested breeding areas were available in the Fish Creek area of the South Saskatchewan.

The field tests were a part of the black fly investigations being carried out from the Dominion Entomological Laboratory at Saskatoon, and the Department of Biology, University of Saskatchewan, and were made with the co-operation of the Suffield Experimental Station of the Defence

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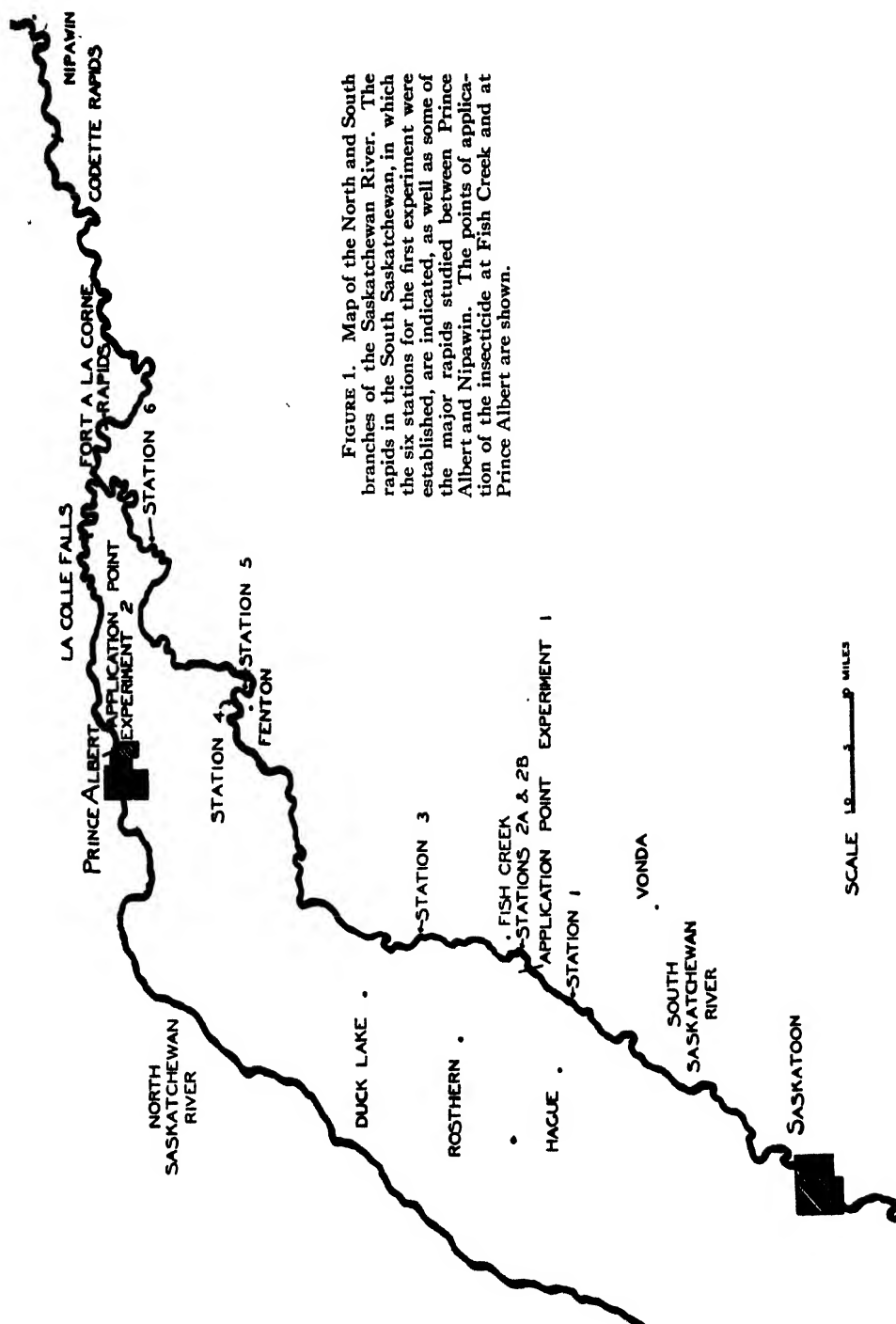


FIGURE 1. Map of the North and South branches of the Saskatchewan River. The rapids in the South Saskatchewan, in which the six stations for the first experiment were established, are indicated, as well as some of the major rapids studied between Prince Albert and Nipawin. The points of application of the insecticide at Fish Creek and at Prince Albert are shown.

Research Board. The latter provided equipment and personnel for the preparation and application of the insecticide, while members of the Saskatoon laboratory and of the University carried out the biological studies.

GENERAL PROCEDURE

The application of DDT in small concentrations has been found effective in the control of black fly larvae in small streams (1). It was decided, therefore, to test the effectiveness of DDT as a larvicide against *S. arcticum* in the Saskatchewan River. Applications by means of aircraft were carried out at Fish Creek on May 25 (Figure 2), and at Prince Albert on June 1. At this time 85 per cent of the larvae were in the first or second instar.

The spray consisted of a 12 per cent solution of DDT (technical grade) dissolved in one part of Velsicol AR50 (an alkyl-naphthalene solvent) and nine parts of fuel oil No. 2. The solution also contained 0.5 per cent of Triton X-100 emulsifier to facilitate its dispersal on the river water, and 0.5 per cent of Williams red dye so that the spray would be visible and its fate could be followed.

For spray application, 500 gallons of the solution were loaded into two tanks (B-25 jettisonable fuel tanks) mounted in the fuselage of a Dakota (C-47) aircraft. The spray was emitted through a vertical pipe, 4 inches in diameter, projecting 18 inches below the belly of the aircraft into still air outside the slipstream. The average rate of emission was three gallons (Imperial) per second, with the aircraft flying at the set speed of 150 miles per hour. This is equivalent to 1 gallon for every 25 yards of flight. The spray was broken up on emission into droplets with a wide range of sizes, whose median diameter (by mass) was approximately 300 microns (0.3 mm.).

Applications were performed in the early morning, while the air was stable and there were no upward convection currents. The emission runs were made across the river on a track slanted diagonally in order to allow a convenient length of run. A number of runs were made over the same track established by ground markers (luminescent fabric panels), dictated by the dosage required. The river flow ensured that a new stretch of water was presented each time for reception of the spray. Emission runs were made every two to three minutes, the time required for the aircraft to make a complete circuit. Instructions as to height of flight, duration of emission, and number of runs, were relayed to the aircraft by radio from the ground.

Samples of the river water, each of two litres, were taken a few miles downstream from the point of application. The red dye in the solution served to indicate when the treated water was passing the sampling point. Water markers of powdered aluminum were also used, but were found to be ineffective for this purpose. The samples were taken just below the surface, and at a depth of four feet, at points established in midstream, as well as close to either bank. Subsequently, they were evaporated to dryness, the DDT was extracted with acetone, and the amount present determined by the Schechter-Haller method.

To assess the results of each treatment, sampling stations were established in the rivers prior to the applications. Each station consisted of a staked transect across an infested rapid. Four quantitative stations and three qualitative stations were set up in the South Saskatchewan River. Figure 1 indicates the location of these stations. The pre-application surveys of these stations were made two and three days prior to the application. Post-application surveys were made 1 day and 16 days after the application.

For the second experiment, in the North branch, 5 stations were established (Figure 1). Two of these were above the junction with the South Saskatchewan, and 3 below the junction, the most distant being 107 miles down river from the point of application at Prince Albert. In this experiment the stations were also sampled just prior to the application. The post-treatment surveys were made 1 day and 4 days after the application. In this case, however, adequate sampling was difficult because of the unusually high level of the river.

At each quantitative station the black fly population was sampled by collecting 30 to 50 rocks from depths of 6 to 30 inches along the staked line. Direct observation being impossible, because of the siltiness of the river, rocks were collected by hand in the shallow water. In the deeper water the collections were made with the aid of an anchored boat and a modified Peterson dredge. After each collection was completed, the rocks were washed off, the black fly larvae and other organisms were allowed to settle out, and then the superfluous water was decanted off. The samples were preserved in formalin. The estimates of larval populations in the quantitative samples were made in the laboratory. In those cases where the populations were too large for a direct count, a portion of the sample was selected at random, counted and weighed. The remainder of the sample was then weighed and the number of larvae in the whole sample estimated. A uniform dryness for the weighed samples was obtained by drying the larvae just to the point where they no longer produced a wet mark on dry filter paper. Since the total area which the sample represented was known, it was then possible to determine the population density per square foot. No attempt was made to sample organisms in places other than those suitable for black fly larvae. The results, therefore, cannot be considered indicative of the effect of the treatment on insects living on silt and sand bottoms.

The possible effect of DDT on fish is of importance. A small amount of angling and netting is done along the entire river. The goldeye (*Amphiodon alosoides* Raf.) is of considerable economic importance. The effect of the field applications on native river fish: pickerel (*Stizostedion vitreum* (Mitch.)), common sucker (*Catostomus commersonii* (Lac.)), river chub (*Platygobio gracilis* (Rich.)), ling (*Lota maculosa* (Le Sueur)), and goldeye, was studied by members of the Department of Biology of the University of Saskatchewan. Field tests in the spring indicated that concentrations of DDT as high as 25 p.p.m. for 30 minutes had no noticeable effect upon the common river fish.



(Photograph of aircraft applying DDT)

FIGURE 2. Aircraft (C47) applying DDT to the South Saskatchewan River at Fish Creek. The direction of flight is indicated by the two markers. The river and river valley are typical of the Saskatchewan River. Stations 2a and 2b are located in rapids at the point on the right bank visible in the distance.

EXPERIMENT ON THE SOUTH SASKATCHEWAN RIVER

The river channel of the South Saskatchewan River has a uniform width and a steady gradient. There are no important tributaries in the area involved in this experiment, nor does the river flow through any lakes or marshes. At the time of the spray application the water temperature was 61° F., with a pH of 7.28 and total solids of 13.9 p.p.m.

The application of the insecticide at Fish Creek (Figure 2) was carried out on May 25, 1948, between 8.10 and 8.46 a.m. The sky was clear and the wind was blowing downstream at speeds from 2½ to 4 miles per hour. The river at this point was 400 yards wide; the emission track was slanted diagonally across the river to give a water distance of 700 yards. The spray was emitted in 16 runs, each of 9 seconds' duration, made at a height of 150 feet above the river. During the total emission period of 144 seconds, 440 gallons were emitted over the river, containing 615 pounds of DDT.

The volume flow of the river at the point of application, as determined from records supplied by the Dominion Water and Power Bureau, was 35,220 cubic feet per second, equivalent to 13.2 million gallons per minute. Therefore, during the 36 minute period of application, 474 million gallons (4740 million pounds) of water were treated. The application to it of 615 pounds of DDT was, therefore, equivalent to 0.13 parts of DDT per million parts of water.

The speed of the water in the middle of the river was 3.5 miles per hour. Thus, in the 2½ minute interval between successive emission runs it flowed 300 yards downstream. The width of the swath of spray deposited was about 100 yards, leaving 200 yards of clear water between each swath. However, the swaths were observed from the air to fuse at the first rapids two miles downstream, and thereafter to move down the river as a continuous film, broken only in small spots as boils of water came to the surface.

The treated water first arrived at the sampling point, located at Fish Creek Ferry five miles downstream, 80 minutes after application was commenced. It continued to flow past for a period of 60 minutes, indicating that its length was 3.5 miles, as compared with the 2.1 mile length of water treated. Thus in travelling 5 miles the added material is diluted by a factor of 1.7. The content of DDT in the water, tabulated below, varied from 0.01 to 0.09 parts per million, the highest concentration occurring at the forward end of the film and in the middle of the river.

TABLE 1.—CONTENT OF DDT IN P.P.M. IN RIVER, FISH CREEK FERRY

Time of sample	North bank		Midstream		South bank	
	Surface	Deep	Surface	Deep	Surface	Deep
0935 hr.	0.012	Trace	0.058	0.085	0.000	0.000
0955 hr.	0.017	—	0.044	0.019	0.017	0.011
1010 hr.	0.018	0.020	0.017	0.016	0.020	0.026
0900 hr.	Control sample taken before passage of insecticide: 0.00					

TABLE 2.—THE EFFECTS OF DDT APPLICATION ON POPULATIONS OF BLACK FLY LARVAE IN THE SOUTH SASKATCHEWAN RIVER ON MAY 25, 1948

Station	Depth (inches)	Distance from point of application	Larvae per square foot		Per cent reduction	Per cent survival*	Larvae per square foot June 10
			May 23 and 24	May 26 and 27			
1	0-30	4.9 miles upstream	5649	3010	46.7	—	—
2a	12-24	1.25 miles downstream	3390	46	98.8	2.6	159
2b	8-24	1.25 miles downstream	8840	11	99.9	0.2	353
3	8-30	17 miles downstream	1780	2	99.9	0.2	42
4	8-30	56 miles downstream	Not examined before application	0	—	—	3
5	6-30	58 miles downstream	Very heavy concentration of larvae on willow stems before application	0	100	0.0	—
6	6-26	92 miles downstream	Not examined before application	Trace	—	—	—

* Percentage survival = $\frac{A_2 C_1}{A_1 C_2} \times 100$ (Hoffman & Surber, 1945),
 when A_1 = pre-treatment population in treated area
 A_2 = post-treatment population in treated area
 C_1 = pre-treatment population in check area
 C_2 = post-treatment population in check area

TABLE 3.—THE EFFECTS OF DDT APPLICATION ON ARTHROPODS OTHER THAN BLACK FLY LARVAE IN THE SOUTH SASKATCHEWAN RIVER ON MAY 25, 1948

Station	Depth (inches)	Distance from point of application	Population per square foot		Per cent reduction	Per cent survival†	Population per square foot June 10
			May 23 and 24	May 26 and 27			
1	0-30	4.9 miles upstream	0.52	0.58	—	—	—
2a	12-24	1.25 miles downstream	0.58	0.08	86.2	12.3	—
2b	8-24	1.25 miles downstream	0.40	0.09	77.5	20.1	0.45
3	8-30	17 miles downstream	0.29	0.27	6.9	83.5	0.16

† Calculated as for Table 2.

Virtually complete disappearance of black fly larvae was observed at the Fish Creek Rapids by the time all the treated water had passed this point. A thorough examination four hours later revealed only an occasional live larva where there had been immense numbers prior to treatment. The effects of the treatment on the black fly larvae are presented in Table 2.

The data obtained from Stations 2a, 2b, and 3 show that the larvae were almost completely eliminated from the river for a distance of 17 miles downstream from the point of application. There were also strong indications that the insecticide was effective at much greater distances as shown by the data obtained at Stations 4, 5 and 6. At Stations 4 and 5, located in the Fenton Rapids over 50 miles below Fish Creek, not a single larva could be found two days after the application. Nine days earlier submerged willows at Fenton were exceptionally heavily infested. Long strands of twisted silk were all that remained to indicate that larvae had been present. At station 6 over 90 miles downstream from Fish Creek, only four larvae were found in a two-hour search. Unfortunately no pre-application survey had been made at this station. It would appear to be significant that two days following the application of DDT at Fish Creek no black fly larvae were found in any rapids below the point of treatment as far as the junction with the North branch, a distance of more than 100 miles. These are the rapids that are normally severely infested with larvae that contribute heavily to the mass flights every spring. Collections below the junction indicated that the dilution had rendered the insecticide ineffective here.

It should be pointed out that the reduction in population shown in Table 2 for Station 1 is believed to reflect a sampling error, rather than an actual change. Sampling at this location was rendered difficult because of the large size of rocks. Even when allowance is made for a natural reduction of this magnitude at the control station, the degree of effectiveness is virtually unchanged.

Within two weeks after the application of the insecticide at Fish Creek, the rapids in the upper end of the cleared section of the river became repopulated with small numbers of mature black fly larvae. Most of the larvae originated in the heavily infested rapids up river from the point of application. On July 27, rapids at Fish Creek and at Fenton, where Stations 2a, 2b and 5 had been located, were re-examined. The entire treated section of the river was found to be populated with second generation larvae. At Fish Creek, where the infestation was heaviest, the population was counted as 4 pupae and about 100 larvae per square foot. At Fenton the examination was the first thorough work of the year, because of the unusually high water level which had prevailed earlier in the season. Previous examinations had been confined to the submerged willows near the banks. On July 27 the river level was considerably lower than at any previous time since the ice break-up and wide areas of the rock beds were exposed. A small number of empty black fly cocoons was found on the prominent boulders in the area. There were approximately 30 of these large boulders exposed in the rapids and they contained fewer than 100 empty cocoons per square foot. Very few pupae were found on the countless number of smaller boulders. From these observations it was evident that there had been considerably less pupation in these rapids than in the previous year.

The elimination of black fly larvae from all of the main breeding areas in the South Saskatchewan River may have prevented an outbreak of major proportions during the spring of 1948. In June, when the remainder of the first generation of black flies had almost entirely emerged, very few black flies could be found on live stock in the vicinity of the treated portion of the South Saskatchewan, whereas the usual large swarms could be found attacking live stock adjacent to untreated portions of the river at Saskatoon and Vonda. Extensive black fly flights were absent for the first time in five years.

The effect of the treatment on aquatic arthropods, other than black fly larvae, was observed immediately after the application. Small numbers of Plecoptera and Ephemeroptera nymphs were found floating in the oil film which lingered in the back-waters below Fish Creek. The data in Table 3 show the extent to which these aquatic insects were affected by the treatment. Their numbers were reduced by 86 per cent and less, near the point of application, and by 7 per cent at a distance of 17 miles downstream.

Laboratory experiments completed later in the year provided additional information as to the effect of DDT on some of these aquatic organisms. Amphipods (an important source of food for fish in the Saskatchewan River) were permanently paralyzed by two-hour exposures to oil emulsions of DDT in concentrations as low as 0.05 p.p.m. With thirty-minute exposures no disabling effects were observed at concentrations less than 0.5 p.p.m.

The fish, of several species, which were confined in cages a short distance downstream from the point of application, or which were observed free in the river, were found to be entirely unaffected by the treatment. Fuller details on this point are given in a report (3) by D. S. Rawson, Department of Biology, University of Saskatchewan.

No dead fish were discovered anywhere during the black fly sampling work. The data obtained from experiments at this laboratory confirmed these observations. Using a DDT suspension in water, three species of river fish were entirely unaffected by the strongest treatment used, namely 25 p.p.m. for 30 minutes. The fish were also unaffected by DDT oil emulsion at the highest concentration, namely, 30 p.p.m. for 15 minutes. Additional experiments indicated that two species of river minnows were unaffected after an exposure of 30 minutes to concentrations as strong as 50 p.p.m. of technical grade DDT as a water suspension.

EXPERIMENT ON THE NORTH SASKATCHEWAN RIVER

The physiography of the area (Figure 1), which is involved in this experiment, is similar to that of the South branch. The water temperature at the time of the experiment was 64° F. The pH and total solids were estimated to be similar to those on the South branch.

The insecticide was applied to the North Saskatchewan River at Prince Albert on June 1, 1948, from 8.23 to 8.57 a.m. The sky was clear and the wind was blowing obliquely across and down the river at 2 to 5 miles per hour. The river at this point was 500 yards wide; the emission track was angled slightly to give a distance of 700 yards. The spray was

emitted in 12 runs, each of 9 seconds' duration, at a height of 120 feet. During the total emission period of 109 seconds, 390 gallons were emitted, containing 545 pounds of DDT.

The volume flow of the river at the point of application was determined to be 63,900 cubic feet per second, or 23.9 million gallons per minute. Therefore, during the 34 minute period of application, 8100 million pounds of water were treated. The application of 545 pounds of DDT was, therefore, equivalent to 0.07 parts DDT per million parts of water.

The speed of the river was 2.1 miles per hour in midstream. In the interval between successive runs it flowed 180 yards downstream. The width of each swath of spray deposited was 40 yards. The series of swaths moved downstream as a succession of V's, due to faster movement in midstream. The swaths were observed to join into a continuous film at the weir $1\frac{1}{2}$ miles downstream from the point of application.

The treated water first arrived at the sampling point 3 miles below the point of application 55 minutes after the first emission run. It continued to flow past for 45 minutes, indicating that it was 1.5 miles long, as compared with 1.2 mile-length treated. Owing to the low content of DDT and the high content of suspended material, accurate determination could not be made of the water samples.

In this field test the application of DDT at the reduced rate of 0.07 p.p.m., over a period of 34 minutes, apparently had little effect on the black fly larvae. Unfortunately, it was impossible to establish satisfactory stations in heavily infested rapids as was done on the South branch, because of the extremely high level of the river. However, the observations at the stations which were set up indicated that there were no significant changes as a result of the application. Fish were also unaffected by this application (3).

DISCUSSION AND CONCLUSIONS

The results of experiments conducted in the Saskatchewan River indicate that an oil solution of DDT, applied at the rate of 0.13 p.p.m. for a period of 36 minutes, resulted in the complete elimination of the larvae of *Simulium arcticum* for a distance of at least 17 miles. There is evidence that the treatment was effective for a distance of 90 miles. On the other hand, an application of 0.07 p.p.m. for a period of 34 minutes proved ineffective. These results are in general agreement as to time and dosage with those obtained at Churchill in 1947 (1). In the South Saskatchewan River experiment it was demonstrated that the concentration of DDT became progressively lower as the distance increased from the point of application. At the same time the exposure period progressively increased with the distance. This would explain how black fly larvae were successfully eliminated in the South branch at the greater distances from the point of application.

The rapids in the upper end of the treated section of the river became lightly repopulated with mature black fly larvae within two weeks after the application. This was the result of a migration of larvae downstream from infested rapids above the point of application. Later in the season the entire treated section of the river became repopulated with second

generation larvae. Few adults, however, were collected during the summer months in the vicinity of the treated section of the river. This is the area which in recent years had suffered heavy live stock losses due to black fly outbreaks.

The effects on the black fly larvae of the inert components in the insecticide were studied in a small-scale field experiment. The results indicated that the DDT was the principal active ingredient in the insecticide.

From the results obtained in these tests it may be concluded that practical control of *Simulium arcticum* may be secured by the application of DDT to the Saskatchewan River in sufficient quantity to produce a concentration of not less than 0.1 p.p.m. for a period of over half an hour. A lower concentration without a corresponding increase in the exposure time would be ineffective. At the effective dosage level virtually complete elimination of larvae in the river for a distance of at least 17 miles and possibly for a much greater distance would be possible. Therefore, one application at Prince Albert on the North branch, and two or three along the South branch down river from Saskatoon, should be sufficient to eliminate infestations from all the main breeding areas from which destructive outbreaks have originated in the past.

Such treatments would be non-lethal to fish. River fish showed no ill effects from either of the two spray applications, and laboratory tests indicated that several species of fish native to the Saskatchewan River could withstand dosages which were several times greater than those which were effective against the black fly larvae. Aquatic insects, other than black fly larvae, were affected during the field tests by the DDT, but to a lesser degree. Their survival may, in part, be attributable to habits which provided them with greater protection from contact with the insecticide.

The cost of this method of black fly control is well within practical limits, in view of the fact that a lethal dosage may be established with a relatively small quantity of insecticide, and that a single application is effective for a considerable distance in the river. It should be pointed out that the volume discharges of the two branches of the Saskatchewan River at the time of the applications were three and seven times greater than average. Thus the amount of insecticide normally required would be considerably less than that used in these control tests.

SUMMARY

Virtually complete clearance of a large river, the South Saskatchewan, of larvae of *Simulium arcticum* Mall., for a proven distance of 17 miles, was obtained by the application of sufficient DDT in an oil solution from an aircraft to give a calculated concentration of 0.13 p.p.m. for a period of 36 minutes. There is evidence that the lethal effect of this application extended down river for 90 miles. Other aquatic insects were affected to a lesser degree. Fish were entirely unharmed. The application of 0.07 p.p.m. of DDT to a similar river, the North Saskatchewan, had little effect on the black fly larvae.

ACKNOWLEDGMENTS

The two field experiments recorded in this paper involved the co-operation of several departments. These are mentioned elsewhere.

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TESTING CEREAL VARIETIES FOR DORMANCY¹

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Dormancy, or "after ripening"* of grain, is important in those regions where prolonged moist weather conditions may occur at harvest. Large losses have been sustained on the plains of Western Canada when wet harvest weather has caused sprouting in swathed and stooked grain awaiting threshing. To protect the farmer and others dependent upon agriculture from these losses it is desirable to have cereal varieties which will remain dormant for several weeks after maturity regardless of the favourableness of the circumstances for germination. Varieties differ very markedly for dormancy and accurate tests are available for the determination of their comparative merits. However, the plant breeder needs tests which are not only accurate but cheap and easy to make. In 1947 and 1948 methods of testing dormancy were studied at Saskatoon with a view to possible simplification.

REVIEW OF LITERATURE

Differences among wheat varieties in their tendency to sprout in the swath or stook have been reported by Deming and Robertson (2), Akerman (1), Feekes (3), Harrington (5), Greer and Hutchinson (4) and others. Harrington and Knowles (6, 7) in 1937 and 1938 studied the dormancy of 110 varieties and hybrid lines of wheat and barley and found large varietal differences in both crops. They also developed a laboratory technique which gave results closely similar to those secured under field conditions at Saskatoon in 1938. They showed that dormancy was an inherited character probably governed by more than one pair of factors.

Hutchinson, Greer and Brett (8), working with wheat, reported in 1948 that they found varietal differences both in seed and ear sprouting at different dates after maturity and they concluded that the ear exerts a restrictive influence on the germination of the seed. Smith (9), also in 1948, showed that the presence of the outer glumes may retard germination considerably.

MATERIALS AND METHODS

Tests in 1947

In the fall of 1947, dormancy tests were made on 62 varieties of spring wheat, 7 of winter wheat, 26 of oats and 40 of barley using a modification of the Harrington-Knowles (6) procedure. Each variety was sampled in the field, sufficient heads at the "just ripe stage"*** being taken at random from border rows of the replicated micro-plot yield tests to furnish material for duplicate 25 seed tests at each of 5, 10, 20, 40 and 60 days after maturity. Where tests of both unthreshed and threshed seeds were desired twice as many heads were harvested. The method of sampling was such as to insure a fair comparison of varieties.

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* "After ripening" is a state of dormancy of the embryo of the mature cereal seed in the presence of conditions favouring germination and is used synonymously with the term "dormancy" in this paper.

** When the kernel is indented with difficulty by the thumb nail.

The head material harvested from the field was stored on shelves in the greenhouse basement from maturity until required for germination tests. The storage temperature was close to $19^{\circ}\text{C.} \pm 2^{\circ}$ and the humidity varied between 68 per cent and 88 per cent. Half of the heads were used for the unthreshed seed tests and half for the threshed seed tests where both were made. In the unthreshed wheat seed tests 3 or 4 half heads were used for each duplicate sample. In making each test duplicate lots each containing 25 seeds (excepting in some instances where the portions of heads in the unthreshed seed test had one or two seeds more or less than 25) which had been ripe for the desired number of days, were placed on inch-deep beds of moist river sand on the greenhouse basement floor where the average temperature was about 18°C. The seeds were then covered with several layers of moist muslin cloth. Water was added daily as required. Readings on germination were made at 5, 10 and 15 days after the start of each test.

All of the varieties of spring wheat were given an unthreshed seed test and seven of them also had a threshed seed test; the winter wheats were all tested both threshed and unthreshed; 13 oat varieties were tested both threshed and unthreshed, but the other 13 varieties only in the unthreshed condition; all of the barleys were tested threshed.

Summarization of the data was effected on the basis of considering germination in excess of 40 per cent as constituting emergence from dormancy. This arbitrary dividing line was checked against others such as 30 per cent and 50 per cent and it was concluded that 40 per cent was the most satisfactory figure to use.

Tests in 1948

The 1948 germination tests were made on both threshed and unthreshed seed of 12 varieties of spring wheat and 8 varieties of oats, and on threshed seed of 8 varieties of barley. The procedure of sampling, testing and summarizing was the same as in 1947 with the following exceptions. For each test alternative spikelets were removed from each of ten heads. Half of the spikelets were used for a duplicate test of unthreshed seeds and the other half for a duplicate test of threshed seeds. After the seed was placed on the moist sand it was covered with a square of moist blotting paper containing the number of the sample. Over these were spread several thicknesses of moist muslin cloth. Germination tests were started on all samples at 5, 10, 20, 30, 40, 50 and 60 days after maturity and for wheat at 70 days in addition. Readings on the threshed seed tests were made at 3, 5, 8, 10 and 15 days after the start of a test. For the barley and oat tests the lots of seed were placed for germination at 5, 10, 20, 35; 50 and 60 days after maturity.

In addition to the use of moist sand beds on a basement floor extensive trials were made using Petri dishes with moist sand or blotting paper in our Cereal Breeding laboratory at temperatures of 19° to 24°C. These tests were not satisfactory, one of the main disturbing factors being moulds. The basement tests in river sand were free from mould interference.

RESULTS IN 1947

The nature and range of the dormancy results are illustrated by the percentage germination data on eight varieties as given in Table 1. For example, Garnet wheat, a variety with very little dormancy, germinated 40 per cent when the unthreshed seed was kept dry for 5 days after maturity and then was placed in moist sand for 10 days, whereas Apex did not germinate as much as 40 per cent until the unthreshed seed had been ripe for 60 days and then was kept in moist sand for 15 days.

TABLE 1.—DORMANCY DATA ON UNTHRESHED SEEDS OF EIGHT OF THE 62 SPRING WHEAT VARIETIES TESTED IN 1947, TO ILLUSTRATE THE NATURE AND RANGE OF THE RESULTS

Days after maturity	Days after start of test	Germination percentages of varieties							
		C.A. 11-101	Apex 1789	Thatcher	Marquis	Regent	Garnet	Rescue	Reliance
5	5	0	0	0	0	0	27	0	0
	10	0	0	1	0	0	40	2	0
	15	0	1	9	0	0	47	6	1
10	5	0	0	0	0	0	86	0	4
	10	0	0	1	0	2	90	2	5
	15	0	0	3	0	2	90	14	6
20	5	0	0	1	0	0	96	0	0
	10	2	0	2	0	0	99	0	1
	15	4	0	6	0	0	99	5	8
40	5	1	0	49	10	55	—	95	63
	10	6	0	62	26	68	—	95	66
	15	8	0	69	51	78	—	98	66
60	5	2	0	68	16	41	—	—	—
	10	2	22	91	34	81	—	—	—
	15	2	43	93	54	88	—	—	—

TABLE 2.—SUMMARIZED DORMANCY OF UNTHRESHED SEEDS OF 62 VARIETIES OF SPRING WHEAT IN 1947

Variety rank	Number of days of dormancy*			Varieties and hybrid lines†
	5	10	15	
1	60†	60†	60†	Comet-Apex 11; CA 11-101
2	60†	60†	60	Apex 1789
3	60	60	60	Red. Sel. 169; Redman 154
4	60†	60†	40	Marquis
5	60†	60	40	Apex 2156; Reg. Can. 918; Reliance C
6	60	60	40	Apex 2177; Mida; Mida-Cadet 609; 3 lines
7	60†	40	40	Regent × That. 171; CA 11-37; 700 × Apex 2194
8	60	40	40	Thatcher; Saunders; 8 lines
9	40	40	40	Rescue; Reliance; Red Bobs; Regent; 29 lines
10	10	5	5	Garnet

* The number of days which elapsed after maturity before exposure to germination conditions for 5, 10 or 15 days, respectively, resulted in more than 40 per cent germination. A † sign indicates a still longer dormancy, in other words the seed was still dormant after 60 days of dry storage.

† Abbreviations: CA = Comet-Apex; Red. Sel. = Redman Selection; Reg.-Can. = Regent-Canus; That. = Thatcher.

Spring Wheat

The results on the dormancy of unthreshed seed of 62 varieties of spring wheat are summarized in Table 2. The seed of Comet X Apex 11 and Comet X Apex 11 Selection 101 germinated less than 40 per cent even when stored dry for 60 days after maturity and then exposed to conditions favourable for germination for 15 days. Apex, Redman, Selections of Apex, Redman and Reliance, Marquis, Thatcher, Mida, Mida-Cadet C.T. 609 and Saunders all showed long dormancy periods. Reliance, Regent, Rescue and Red Bobs showed from 45 to 55 days of dormancy.

Oats and Barley

Tests on the dormancy of threshed seed were made on 26 varieties of oats and 40 varieties of barley. The results are given in summarized form in Table 3. None of the oat varieties had as long dormancy periods as six of the barley varieties. Among the oat varieties Fortune and Garry showed dormancy of from 40 to 50 days. Vanguard, Valor and Overland ranked next in dormancy. Intermediate in dormancy were Banner, Ajax, Exeter, Beacon and Victory. Parkside and the hullless variety Brighton had the lowest rank with only 10 to 20 days of dormancy.

TABLE 3.—SUMMARY OF DORMANCY DATA ON THRESHED SEEDS OF 26 VARIETIES OF OATS AND 40 VARIETIES OF BARLEY IN 1947

Variety rank	Number of days of dormancy*			Varieties and hybrid lines†
	5	10	15	
<i>Oats</i>				
1	35	35	35	Fortune and Garry
2	20	20	20	Vanguard; Valor; Overland; Legacy; 2 lines
3	10	10	10	Banner; Ajax; Exeter; Beacon; Victory; 2 lines
3	20	5	5	Nakota × C 33-16; Nak.-Haj. 30
4	10	10	5	Nakota × Haj. 25; Nak. Goph. 43
5	10	5	5	Nak. × Haj. 31
6	5	5	5	Parkside; Brighton; 4 lines
<i>Barley</i>				
1	50	50	50	Warrior; TS 14-Peat. 25; Bran. 1360
2	50†	50	35	Glacier
3	50	35	35	Gem
4	50	35	20	MCB 179
5	50	20	20	Velvon 11
6	35†	20	20	Bran. 1166
7	50†	10	10	MCB 163
8	20	20	20	OAC 21; Vantage; Plush 1732; 3 lines
9	20	20	10	Newal; Tregal; MCB 173
9	35	10	5	TS 14-Peat. 16
10	35	5	5	Compana
11	20	10	10	Titan; Plush 1117; Regal; 3 lines
12	20	10	5	Frontier; Ottawa 94
13	10	10	10	Montcalm; Feebar; Bay; 3 lines
14	10	5	5	TS 17-Peat. 10; US 5-37-1
15	5	5	5	UA 46; UA 10; UA 24; TS 14-Peat. 21

* See footnote of Table 2.

† Abbreviations: Nak-Haj = Nakota-Hajira; Goph. = Gopher; TS = Trebi-Sol; MCB = Multiple Cross Barley; Bran. = Brandon; Peat. = Peatland; US = University of Saskatchewan; UA = University of Alberta.

The barley varieties ranking high for dormancy period were Warrior, Trebi-Sol X Peatland 25 (both hooded varieties), Brandon 1360, Glacier and Gem. Next in rank came Velvon 11 and Multiple Cross Selection 179-33. O.A.C. 21, Vantage, Plush, Newal, Tregal, Compana, Regal, Titan and Frontier were intermediate in dormancy. Montcalm, Feebar and Bay showed from 15 to 25 days' dormancy. Four new hybrid varieties, including three University of Alberta selections, had the lowest dormancy.

Threshed versus Unthreshed

Tests of both unthreshed and threshed seeds were made on 5 varieties of spring wheat, 7 of winter wheat and 13 of oats. The results are summarized in Table 4. In general the dormancy period of a variety was considerably longer when it was tested unthreshed. However, in some varieties there was little difference. In fact, Garnet spring wheat, Kharkov winter wheat and Ajax oats sprouted equally well whether threshed or unthreshed. This variation among varieties as to the protective influence of the chaff was not unexpected in view of results obtained by Luther Smith (9).

TABLE 4.—DORMANCY RESULTS ON THRESHED AND UNTHRESHED SEEDS OF 12 VARIETIES OF WHEAT AND 13 VARIETIES OF OATS IN 1947

Variety	Unthreshed seeds				Threshed seeds			
	Number of days of dormancy*			Variety rank	Number of days of dormancy*			Variety rank
	5	10	15		5	10	15	
<i>Spring Wheat</i>								
CA 11-2139	60†	60†	60†	1	60	40	40	1
CA 11-101	60†	60†	60†	1	40	40	40	2
Apex	60†	60†	60	2	60	40	40	1
Reliance	40	40	40	3	40	20	20	3
Garnet	10	5	5	4	20	10	5	4
<i>Winter wheat</i>								
Kanred	40	40	20	1	10	10	5	2
McAlpine	40	20	5	2	5	5	5	3
Wh-Ry. Hyb.	40	5	5	3	5	5	5	3
Yogo	20	10	10	4	20	5	5	1
Dawson	20†	10	5	5	5	5	5	3
Buffum	10	5	5	6	5	5	5	3
Kharkov	5	5	5	7	5	5	5	3
<i>Oats</i>								
Fortune	65†	65†	65†	1	35	35	35	1
Garry	65†	65†	65†	1	35	35	35	1
Victory	65†	65†	65†	1	10	10	10	3
Exeter	65†	65†	65†	1	10	10	10	3
Vanguard	65	65	65	2	20	20	20	2
Banner	50†	50†	50†	3	10	10	10	3
V.C. 15	50	35	35	4	20	20	20	2
Valor	35	35	35	5	20	20	20	2
Beacon	65†	10	10	6	10	10	10	3
Brighton	20	20	20	7	5	5	5	4
Legacy	20	10	10	8	20	20	20	2
Ajax	10	10	10	9	10	10	10	3
Parkside	10	10	5	10	5	5	5	4

* See footnote of Table 2.

The dormancy rating of the varieties was not the same for unthreshed as for threshed seed tests. In the spring wheat varieties there was a very large range of dormancy and the "threshed" ranking for dormancy is almost identical with the "unthreshed" ranking. In the winter wheat results the variety Yogo is out of line in that it had an intermediate rating in the unthreshed seed test and a high rating in the threshed seed test. In addition, Dawson, Buffum and Kharkov all rated equally low in the "threshed" test but were clearly differentiated in the "unthreshed" test. In the oat results, Victory and Exeter ranked high for dormancy when unthreshed but only intermediate when threshed. In fact, Ajax, which ranked very low unthreshed, was as high as Victory and Exeter when threshed.

RESULTS IN 1948

The 1948 tests were run for the purpose of improving on the testing technique; consequently relatively few varieties were used.

Spring Wheat

The summarized results on 12 varieties of wheat are given in Table 5. Unthreshed seed of Comet \times Apex 11 Selection 101 and Comet \times Apex 11 Selection 19, Redman, Apex 2177 and Saunders remained dormant for 55 to 75 days after maturity. Garnet, Reliance and Red Bobs showed the shortest dormancy periods and the other varieties were intermediate. On the whole the "unthreshed" and "threshed" results agree very well as to varietal rank for dormancy. In general the unthreshed seed retained its dormancy approximately 20 days longer than did the threshed seed. All varieties showed a 5-day unthreshed seed dormancy when tested 5 days after maturity and all but Red Bobs retained their dormancy for 10 days in this test. Garnet and Reliance were the only varieties to lose their dormancy in 5 days when tested 10 or 20 days after maturity.

TABLE 5.—DORMANCY RESULTS ON THRESHED AND UNTHRESHED SEEDS OF 12 VARIETIES OF SPRING WHEAT IN 1948

Variety or line	Unthreshed seeds				Threshed seeds					
	Number of days of dormancy*			Variety rank	Number of days of dormancy*					Variety rank
	5	10	15		3	5	8	10	15	
CA 11-101	70	50	50	1	30	30	30	30	30	2
CA 11-19	70	50	50	1	40	30	30	30	5	1
Redman	60	40	40	2	20	20	20	20	20	4
Apex 2177	50	50	40	3	20	20	20	20	20	4
Saunders	50	40	40	4	30	30	30	30	30	3
Thatcher	40	30	20	5	20	20	10	5	5	6
Hope-Tim	40	10	5	6	20	20	5	5	5	7
Marquis	30	30	30	7	20	20	20	20	10	5
Rescue	30	20	5	8	20	10	10	10	10	8
Red Bobs	30	5	5	9	20	5	5	5	5	10
Garnet	20	20	10	10	20	10	5	5	5	9
Reliance	10	5	5	11	10	5	5	5	5	11

* See footnote of Table 2.

Oats and Barley

Threshed and unthreshed seed of 8 varieties of oats and threshed seed of 8 varieties of barley were tested. The results appear in Table 6. Valor oats ranked at the top for dormancy both in the unthreshed and threshed seed tests. Victory and Fortune were next in rank, while Ajax and Brighton were at the bottom for dormancy. There was definite inconsistency between the "threshed" and "unthreshed" results, yet the tests agreed excellently on varietal ratings. The barley results show Warrior with the top rank, and Trebi next. Montcalm is at the bottom and the other varieties show intermediate positions.

TABLE 6.—SUMMARIZED DORMANCY RESULTS ON 8 OAT AND 8 BARLEY VARIETIES IN 1948

Variety	Unthreshed seeds						Threshed seeds					
	Number of days of dormancy*					Variety rank	Number of days of dormancy*					Variety rank
	3	5	8	10	15		3	5	8	10	15	
<i>Oats</i>												
Valor	60†	60†	40	40	30	1	60	50	50	50	50	1
Victory	60†	20	10	5	5	2	60	5	5	5	5	2
Fortune	60†	20	5	5	5	3	60	5	5	5	5	3
Beacon	60†	5	5	5	5	4	20	5	5	5	5	4
Exeter	60†	5	5	5	5	4	20	5	5	5	5	4
Clinton	60†	5	5	5	5	4	5	5	5	5	5	5
Ajax	30	5	5	5	5	5	5	5	5	5	5	5
Brighton	30	5	5	5	5	5	5	5	5	5	5	5
<i>Barley</i>												
Warrior	—	—	—	—	—	—	60	60	60	50	40	1
Trebi	—	—	—	—	—	—	60	40	30	30	20	2
Bran. 1360	—	—	—	—	—	—	40	40	40	20	20	3
Plush 1732	—	—	—	—	—	—	20	20	20	20	20	4
Newal	—	—	—	—	—	—	20	20	20	20	10	5
Vantage	—	—	—	—	—	—	20	10	5	5	5	6
Titan	—	—	—	—	—	—	10	10	10	10	10	7
Montcalm	—	—	—	—	—	—	5	5	5	5	5	8

* See footnote of Table 2.

SUMMARIZED RESULTS FOR MORE THAN ONE YEAR

The wheat results for 1947 and 1948, along with those obtained in 1937 and 1938 by Harrington and Knowles (6) are reasonably consistent for the five varieties tested in both periods. Apex averaged the highest in dormancy and for any two-year average ranked higher than either Marquis or Thatcher, which in turn ranked much higher than Reliance or Garnet.

Twelve wheat varieties were tested in both 1947 and 1948. (Tables 2 and 5). The results showed CA 11-101, Apex 2177, Redman, Saunders and Thatcher to have a longer dormancy period than Rescue, Hope-Timstein and Red Bobs. Marquis and CA 11-19 were intermediate in that they were not consistently with either of the two groups. Reliance and Garnet constitute the lowest group with Garnet definitely at the bottom.

Tests for both 1947 and 1948 are available for eight barley varieties (Tables 3 and 6). The results agree as to varietal ranking as follows: Warrior, Brandon 1360, Plush, Newal, Vantage, Titan and finally Montcalm at the bottom.

The oat varieties Fortune, Victory, Garry, Valor, Exeter, Beacon, Ajax and Brighton were tested in both 1947 and 1948 (Tables 3, 4 and 6). The ranking of these varieties in the unthreshed seed tests was fairly consistent in the two years, Brighton and Ajax being at the bottom and Fortune, Garry and Victory being at the top. Valor, however, varied from an intermediate variety in 1947 to the top in 1948. The threshed seed results were less consistent than the unthreshed in differentiating the moderately long and long dormancy varieties.

RETURN TO DORMANCY IN OATS

In the 1947 oat tests, and to some extent in the 1948 tests, there were clear-cut instances of return to dormancy. In 1947, nine out of 26 varieties tested as threshed seed and eight of these plus two others, when tested as unthreshed seed, showed return to dormancy. In 1948, out of eight varieties one showed return to dormancy in the threshed seed test and three showed this tendency in the unthreshed seed test. This phenomenon is illustrated with a portion of the 1947 data in Table 7.

Return to dormancy was striking in some varieties and not apparent in others. Threshed seed of Victory had "emerged" (germinated over 40 per cent) from dormancy 10 days after maturity, then made a partial return to dormancy by 20 days after maturity. At 35 days after maturity Victory was again out of its dormancy but there was a tendency toward a return to dormancy 50 days after maturity, which, at 65 days after maturity, had materialized. Yet unthreshed seed of Victory showed no tendency to return to dormancy. Threshed seed of Valor emerged from dormancy 20 days after maturity but at 50 days after maturity showed some tendency to return to dormancy. Beacon showed a definite return to dormancy in the unthreshed seed test 20 days after maturity. Banner shows a striking return of dormancy in the threshed seed test 20 days after maturity and a less apparent return at 65 days after ripening.

DISCUSSION

The need for conducting dormancy tests on cereal breeding material is again affirmed by the data obtained in the present study. In both years and with all three crops, the varieties ranged from short to long dormancy. Commercial field experience with short dormancy varieties, such as Garnet wheat, has demonstrated that those varieties are as unreliable and likely to cause large economic loss as are varieties which shatter readily or are susceptible to a disease which occasionally assumes epidemic proportions. It is desirable for a cereal breeder to make dormancy testing a part of his testing program so that he will not release a new variety which does not possess at least a moderately long dormancy period. Indeed, it would seem that the cereal breeder should consider dormancy as an important character in his breeding program. In each of the crops, wheat, barley and oats,

TABLE 7.—GERMINATION DATA ON BOTH THRESHED AND UNTHRESHED SEED OF SIX OF THE VARIETIES OF OATS GROWN IN 1947 IN WHICH A RETURN OF DORMANCY WAS FOUND

Days after ripe	Days after test starts	Germination percentages of varieties											
		Unthreshed seeds						Threshed seeds					
		Banner	Victory	Fortune	Valor	Beacon	Garry	Banner	Victory	Fortune	Valor	Beacon	Garry
5	5	14	0	0	0	6	1	14	4	2	0	16	4
	10	19	0	0	0	11	9	16	4	2	0	18	6
	15	19	5	0	0	12	12	16	4	2	0	18	16
10	5	31	0	2	0	18	7	64	54	6	0	82	24
	10	31	0	4	3	42	14	64	54	8	2	84	26
	15	32	2	4	7	42	15	64	56	8	8	84	26
20	5	14	1	7	13	6	14	12	28	14	48	36	24
	10	20	1	7	16	6	19	12	28	14	50	46	34
	15	20	1	7	16	6	19	12	28	14	50	46	34
35	5	26	4	2	42	1	13	64	62	46	70	36	52
	10	33	10	8	51	2	15	64	68	52	76	36	52
	15	33	11	8	53	2	15	66	68	52	76	36	54
50	5	4	6	3	49	6	14	48	42	70	52	18	52
	10	9	16	10	54	12	20	48	46	70	54	20	54
	15	9	19	15	54	13	20	50	46	70	54	20	54
65	5	—	23	7	28	11	4	22	26	58	—	64	40
	10	—	33	7	28	20	4	22	26	60	—	72	42
	15	—	33	7	28	21	4	24	26	60	—	72	42

there are varieties available which can supply the genes necessary for long dormancy. Some examples are: Apex and Redman wheat, Warrior and Brandon 1360 barley and Fortune and Garry oats.

It is of particular interest that varieties recently originated by the Field Husbandry Department of the University of Saskatchewan, where satisfactory performance in a dormancy test is considered a required character in the breeding program, rate very highly for length of dormancy period. These varieties are Apex and C.A. 11 wheat, Valor and Fortune oats and Warrior barley.

Effect of Chaff on Germination

The effect of the chaff in retarding germination has been discussed by Smith (9) and by Hutchinson, Greer and Brett (8). The effect was pronounced in the present study in both wheat and oats but not in all varieties. Three of the winter wheat varieties tested in 1947 (see Table 4) sprouted readily when tested as threshed seed but showed several weeks of dormancy when tested with the glumes enclosing the seeds. On the other hand, Kharkov showed no protective effect of the chaff. In oats also the germination-retarding effect of the glumes differed with different varieties in both years. For example in 1947, Banner and Victory (Table 4) show pronounced effects whereas Ajax showed no retarding effect of the glumes. The striking difference in the reaction of Clinton oats in 1948 and of two of the winter wheats in 1947 makes one hesitate to place full reliance on a threshed seed test.

Standard Dormancy Tests

The testing of varieties for dormancy is time-consuming and expensive; consequently any legitimate abbreviation of time or work is highly desirable. Dormancy tests on unthreshed seeds of wheat and oats give a closer approach to actual weathering in the field than do tests on threshed seed. However, the results indicate that threshed seed tests easily separate varieties having short dormancy from those with long dormancy periods. With either unthreshed or threshed seed, the varieties react sufficiently differently in different years (as shown in Tables 2 to 6) to make it advisable to have results from material grown in two seasons before drawing definite conclusions as to variety ranking for dormancy. The results suggest that a dormancy test in a single season, using either unthreshed or threshed seed, provides safe discarding of the varieties with short after-ripening periods. The results further suggest that a two-year test, using either threshed or unthreshed seed, should give a reliable placing of a variety as to whether it has a long, moderately long, short or very short after-ripening period. Since the threshed seed tests are much easier to make and take approximately 20 days less time, it would seem that either a one- or two-year test of threshed seed should be used.

Feasibility of Preliminary Abbreviated Tests

The technique used for testing dormancy has involved starting germination tests at six to eight intervals after maturity and making germination counts thereafter in 5, 10 and 15 days, or oftener. While the information gained is valuable in securing a fairly complete picture of varietal response,

the results of the 1947 and 1948 studies have indicated that a substantially accurate picture of the relative ranking of varieties for dormancy might be obtained with an abbreviated test.

A suitable abbreviated preliminary test for spring wheat conceivably could be a five-day germination test made with seed held for 10, 20 and 30 days after maturity. The seed test 10 days after maturity would be expected to show which varieties have reasonably long dormancy periods and allow elimination of those with short periods. The seed test 20 days after maturity would be expected to pick out varieties with long dormancy periods in some seasons, whereas the test of seeds 30 days after maturity might be better in other seasons.

The efficacy of the proposed tests may be estimated by reference to the available 1947 and 1948 data. The 10 + 5 test (i.e. 10 days after ripe plus 5 days under conditions favourable for germination) would have singled out Garnet, Reliance, Red Bobs and Rescue in 1948 as varieties of short dormancy. The 20 + 5 test in 1948 would have grouped all of the other varieties as being satisfactory. The 30 + 5 test would have grouped C.A. 11-101, C.A. 11-19 and Saunders at the top. The abbreviated tests in question would have proven unsatisfactory for the 1947 wheat tests (see Table 4) since the five-day germination test would have been too short for the spring wheat and too long for the winter wheat varieties. Additional germination readings at three or four and eight or ten days would seem desirable.

An abbreviated preliminary test for oat and barley varieties could be the same as that suggested for wheat with some exceptions. A ten-day germination reading was much more differential than a five-day reading in 1947 on both oats and barley, and with oats in 1948 the three-day reading was more differential than the later readings.

Where it is desired to distinguish further among the longer dormancy varieties of any of the crops, germination readings could also be made at 10 and 15 days with a minimum of extra work. Actually it is of value to do this, since, under field conditions, periods of damp weather may continue for two weeks although usually they would be of much shorter duration.

Summarizing the discussion on abbreviated preliminary tests the following seem appropriate to suggest: For all three crops germination readings at 4 or 5 days, again at 8 to 10 days, and for some varieties also at 15 days, of duplicate samples of seeds stored dry after maturity for 10, 20 and 30 days prior to being tested. The general amount of dormancy may differ in different seasons as shown for oats in 1947 and 1948 (Tables 3 and 6), and at present there is no way of knowing in advance what the particular effects of a given season on dormancy are likely to be. The tests are well along before this information begins to be available. Therefore, while the abbreviated dormancy tests may be valuable as preliminary elimination tests, they cannot replace the more comprehensive tests. In addition the occurrence of return to dormancy in most of the oat varieties might mean that tests for two years should be considered essential to establish the dormancy rating of an oat variety. Nevertheless, the results on oats obtained in this study show that, despite the recurrence of dormancy, an abbreviated test would have separated satisfactorily short dormancy varieties from long dormancy varieties.

Varietal Heterogeneity Respecting Dormancy

The dormancy results in this study, as illustrated by the germination percentages given in Tables 1 and 7, suggest strongly that the varieties of wheat, oats and barley in general farm use are by no means pure breeding with respect to this character. In fact, the data indicate that each variety, with few exceptions, may be a mixture of biotypes. Further work is under way at Saskatoon to check this assumption. Tangible evidence favouring the assumption is the difference in dormancy period between Reliance and Reliance C, a selection with a distinctly longer dormancy than its parent. Other evidence is the wide range in dormancy among 20 lines selected from the unnamed but morphologically uniform variety Comet-Apex 11. Two of the lines, C.A. 11-101 and C.A. 11-19, rank high for length of dormancy whereas other lines have intermediate dormancy and still others rank as low as Reliance, Red Bobs and Rescue (see Tables 2 and 5). It may well be that selection for uniform long dormancy might be worth-while in such varieties as Thatcher and Saunders wheat.

SUMMARY

1. Dormancy tests were made on 62 spring and 7 winter varieties of wheat, 26 of oats and 40 of barley in 1947 and on 12 varieties of wheat, 8 of barley and 8 of oats in 1948 at Saskatoon, Sask.

2. The main purpose of the tests was to improve on existing testing procedures and to devise if possible a suitable abbreviated preliminary test to use on hybrid lines.

3. The tests were made in cloth-covered beds of moist sand on a basement floor with duplicate samples of seed which had been stored for various lengths of time after maturity. Germination readings were made after 5, 10 and 15 days in most cases.

4. Tests were made on both unthreshed and threshed seeds of 12 varieties of wheat and 13 of oats in 1947, and of 12 varieties of wheat and 8 of oats in 1948. All barley tests were made on threshed seed of hulled varieties.

5. The varieties of each crop showed a wide range in dormancy in both years.

6. The results in 1947 agreed well with those of 1948 and there was general agreement between the unthreshed and threshed seed results.

7. Study of the results indicates that an abbreviated test, consisting of germination readings at 4 or 5 days, again at 8 or 10 days, and for some varieties also at 15 days, on seed stored for 10, 20 and 30 days after maturity would be useful as a preliminary test. It is suggested that such a test would clearly separate short and long dormancy varieties in one season, but that to get an accurate ranking of varieties or lines a two years' test would be necessary, especially in oats.

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NEW AND IMPROVED METHODS OF EXTRACTING FAT FROM CHEESE, FRESH CURD AND MILK FOR FAT ACIDITY DETERMINATION¹

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A number of methods have been published for extracting fat for the determination of fat-acidity in cheese. Lane and Hammer (1) accomplished this by submitting a mixture of cheese and sand to pressure, separating the fat, and finally filtering it through a dry paper. Irvine (2) greatly simplified the procedure by leaving ground cheese to oil off in tall beakers set in warm water. The melted butter fat was used directly.

For some time a procedure similar to that of Irvine's has been used by the authors (3). It consists of placing a grated Cheddar cheese sample in a suitable funnel provided with a folded filter paper and supported in a 65° C. oven. At the elevated temperature the cheese fat melts, runs down and filters into a small receiving flask. The temperature selected does not appear to be critical and 100° C. has been successfully used, shortening considerably the filtration period.

While this method was found satisfactory for Cheddar cheese, considerable difficulty was experienced in extracting the fat from fresh cheese curd. In working out a method for extracting fat from fresh curd an improved method was also developed for cheese. The new procedure for extracting the fat from young and aged cheese is as follows: Approximately 250 grams of cheese are ground in a Waring Blendor, transferred to a 600 ml. beaker and heated in the dry state on a boiling water bath until "oiling off" occurs. Sufficient fat (20.0 gm.) for duplicate tests may be obtained in less than thirty minutes of heating. The fat is then transferred to a funnel provided with a filter paper and held at 45° C. until sufficient fat is collected.

In separating the fat from fresh curd, it has been found necessary to further modify existing methods of fat extraction. Approximately 800 grams of curd is taken and divided into portions of a size permitting convenient grinding in a Waring Blendor. Each portion is finely ground in the dry state; then without stopping the blendor, 500 ml. of hot (90° C.) water is added and the whole thoroughly mixed. This process is repeated for each portion. The water-curd mixtures are poured off into beakers and held at 0° C. until a fat layer has been formed. The fat is skimmed off, placed in an Erlenmeyer flask and churned by hand or by a mechanical shaker. The resulting fat is clarified by centrifuging and then filtered.

In order to obtain fat from cheese milk for fat-acidity determination, two quarts of milk at 25° C. are churned in a small Dazey churn until fairly large granules are formed. Sufficient water at 8° C. is added to cool the fat. It is then skimmed off. The fat is then melted, centrifuged and allowed to filter as in the case of fat from cheese or fresh curd.

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Following the isolation of the dry filtered fat, a 10 gm. portion is titrated in boiling 95 per cent neutral ethanol using 8 drops of phenolphthalein indicator. The titration is essentially that which has been deleted from the fifth and sixth editions of *Methods of Analysis* (A.O.A.C.) (4), but is still useful in certain phases of dairy science. The result is expressed as ml. of N/10 NaOH per 10 gm. fat and is termed the acid degree.

SUMMARY

New and improved methods of extracting fat from cheese, fresh curd and milk for fat-acidity determination have been described. A simple method for determining the acid degree of cheese fat has also been included.

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THE CHEMICAL CONTROL OF THE TOMATO HORNWORM
ON TOBACCO IN ONTARIO¹C. J. FOX², G. M. STIRRETT³, D. A. ARNOTT⁴ AND H. B. WRESSELL⁵*Dominion Entomological Laboratory, Chatham, Ontario*

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The tomato hornworm, *Protoparce quinquemaculata* (Haw.), is an important insect enemy of tobacco in Ontario. The larvae feed voraciously on the foliage, and most growers are obliged to spray or dust every year to avoid serious losses. Considerable work has been done on the chemical control of the tomato hornworm on tobacco, the results of which may be of interest to those concerned with tobacco culture. Starting in 1938, experiments have been undertaken by the Dominion Entomological Laboratory, Chatham, Ontario, not only to improve hornworm control but also to find suitable insecticides which would not leave poisonous or otherwise objectionable residues on treated plants. As new insecticides and new methods of control are being developed it seems advisable to bring together the results of these earlier experiments, both for a matter of record and comparison of results.

HISTORY

The first record of hornworm attacking tobacco in Ontario is contained in the evidence taken by the Ontario Agricultural Commission in 1880. J. P. McKinlay of Kent County is quoted as saying, "The tobacco worm was troublesome to the leaves sometimes; and, if it was left alone, would devour a considerable portion of the crop" (1).

Between 1880 and the present, the insect is mentioned irregularly in the reports of the Dominion Entomologist (4), (5), in the Annual Reports of the Entomological Society of Ontario (2), in the Canadian Insect Pest Review (8) and in the Reports of the Dominion Experimental Station, Harrow, Ontario (6), (7). These publications indicate that irregular peaks of high population occurred in the years 1892, 1901, 1919, 1921, 1924, 1931, 1939 and 1945.

Prior to 1920, arsenical sprays were used in years of severe hornworm infestations to replace the older method of hand-picking. Subsequently, the use of acid lead arsenate became general as a regular annual treatment and it then became necessary to consider the poisonous residues of lead and arsenic. Growers were urged to practise other control measures, which

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included fall ploughing, the destruction of larvae in curing barns and on second-growth tobacco, and the trapping of adults. None of these was considered sufficiently effective to warrant the effort and none was generally accepted. The only control considered practical, then as now, is an application of insecticide to the leaves. This is indicated in a recent survey conducted by a large tobacco company in Ontario which disclosed that about 95 per cent of the growers of flue-cured tobacco and about 50 per cent of the burley growers apply insecticides annually.

FIELD EXPERIMENTS

In 1938, a series of field experiments was begun by the staff of the Dominion Entomological Laboratory at Chatham, Ontario, under the direction of G. M. Stirrett. Two of these experiments were conducted at the Norwood farm, Lynedock, Ontario; one at the de Meyere farm, Thamesville, Ontario; and the remainder at the Dominion Experimental Substation, Delhi, Ontario.

In discussing these experiments the term "commercial control" is occasionally used. The term is elastic and differs in meaning when applied to various crops and indeed on the same crop under varying circumstances. In years of low or average hornworm populations, 70 per cent reduction of the larval population on tobacco may be considered "commercially" adequate, but during outbreaks, control would need to approach or exceed 95 per cent to protect the crop adequately. Control of the larvae must, as a consequence, keep severe leaf damage to 3 per cent or less. Observations by competent growers and entomologists have shown that fields with more than 3 per cent severe damage are not considered a commercial success.

The effects of varying strengths of an insecticide and the effects of different insecticides were evaluated by determining the relationship between the number of larvae surviving under the different conditions of treatment and in some cases also by recording the percentage of injured leaves. In the tables given, the figure "percentage control" is based on the population in the untreated check plots.

As hornworm moths continue to oviposit and the eggs continue to hatch after insecticidal treatments, more than one count of the larval population was usually undertaken. In this way, information was obtained on the residual effect of the insecticide on the surviving or newly hatched population. In no case was the evaluation carried to the end of the growing season to determine the full residual effect.

Prior to applying any of the treatments, eggs and larvae were counted on a large sample of plants to determine the proper time to treat the plots. When a total of 5 eggs and larvae were seen on 100 plants it was considered time to begin application. Post-treatment larval counts in the various tests were based on from 5 per cent to 40 per cent of the plants in the experimental areas, depending on the particular experiment.

As a suitable gauge was not available on the sprayers used, records of the pressures at which sprays were applied were not kept, the dosage being based on the total amount per acre and the coverage.

Results of 1938 Experiments

During the 1938 season, tests were made of possible substitutes for lead arsenate, using barium fluosilicate, synthetic cryolite, and calcium arsenate. Treatments, and an untreated control, were randomized in plots and replicated 6 times. Each plot was composed of 11 rows 34 feet long and had an area of about one thirty-fifth of an acre. Treatments were applied on July 26 by means of a wheelbarrow hand-operated sprayer with a capacity of 15 gallons. To measure the effectiveness of the sprays, larvae were counted on 10 per cent of the plants selected at random on July 29 and August 4. The results of the test are given in Table 1.

TABLE 1.—THE RESULTS OF CHEMICAL CONTROL EXPERIMENTS CONDUCTED FOR THE CONTROL OF HORNWORM ON FLUE-CURED TOBACCO PLOTS AT THE DOMINION EXPERIMENTAL SUBSTATION, DELHI, ONTARIO, JULY 26 TO AUGUST 4, 1938

Treatment and rate per acre applied July 26	Number of larvae per treatment		Percentage control vs. check	
	July 2	August 4	July 29	August 4
Lead arsenate, 1 lb.	10	6	89	74
Cryolite, 13 lb.	8	74	58	27
Calcium arsenate, 6 lb.	5	81	43	20
Barium fluosilicate, 13 lb.	82	90	8	11
Untreated check		101	—	—

Table 1 shows that under the conditions of this experiment synthetic cryolite was the best of the substitutes but was not so effective as lead arsenate when both were applied at the rate of 13 pounds per acre. Calcium arsenate and barium fluosilicate resulted in inferior controls at the rates tested.

Results of 1939 Experiments

In 1939, lead arsenate was applied as early, late, and combined early and late sprays. The chief object was to determine if the general practice of applying more than one spray was actually necessary. The term "early" as used in connection with this and a subsequent experiment means as soon as 5 eggs or larvae were seen on 100 random plants; "late" means 1 or 2 weeks thereafter.

Plots of one thirty-fifth of an acre were again used and each treatment was replicated 6 times. Larvae were counted on 10 per cent of the plants on July 28, 14 days after the "early" application and 2 days after the "late" application. The high rate of application in this test was the result of a faulty wheelbarrow sprayer which delivered the spray material at the heavy volume of 150 gallons per acre.

Table 2 indicates that in this experiment an early spray of lead arsenate at the rate of 18.5 pounds per acre was as effective as the combined early and late sprays of 18.5 pounds each; also that a late spray of 18.5 pounds was almost as effective as a combined early and late treatment of 7.4 pounds.

TABLE 2.—THE RESULTS ON JULY 28 OF APPLYING "EARLY" AND "LATE" LEAD ARSENATE SPRAYS, AT VARYING RATES FOR CONTROL OF HORNWORM ON FLUE-CURED TOBACCO PLOTS AT THE DOMINION EXPERIMENTAL SUBSTATION, DELHI, ONTARIO, ON JULY 14 (EARLY) AND JULY 26 (LATE), 1939

Treatment and rate per acre	Total pounds applied per acre	Number of larvae per treatment	Percentage control over check
Early, 18.5 lb.	18.5	7	97
Early and late, 18.5 lb.	37	8	97
Early and late, 7.4 lb.	14.8	19	92
Late, 18.5 lb.	18.5	24	90
Early, 7.4 lb.	7.4	35	85
Late, 7.4 lb.	7.4	64	72
Untreated check	0	229	—

In view of the infestations, all treatments were considered commercially successful except the last 7.4 pounds of lead arsenate per acre applied as a single, late spray.

Tests were also carried out in 1939 using nicotine bentonite, lead arsenate, and synthetic cryolite. The same spray equipment and plot arrangement were employed as in the 1938 experiment. To evaluate the treatments, the larvae were counted on 10 per cent of the plants.

The data in Table 3 were interpreted by those conducting the experiment as showing that lead arsenate applied at the rate of $3\frac{1}{2}$ pounds per acre and cryolite at the rate of 8 pounds per acre gave commercially adequate controls. The remaining treatments, cryolite at 6 pounds per acre and nicotine bentonite at 8 pounds per acre, were not considered satisfactory.

TABLE 3.—THE EFFECT ON THE HORNWORM POPULATION OF APPLYING VARIOUS SPRAY MATERIALS AT 40 GALLONS PER ACRE TO FLUE-CURED TOBACCO PLOTS AT THE DOMINION EXPERIMENTAL SUBSTATION, DELHI, ONTARIO, JULY 20 TO 25, 1939

Treatment	Pounds of poison applied per acre (July 20)	Number of larvae per treatment (July 25)	Percentage control over check
Arsenate of lead	$3\frac{1}{2}$	25	87.5
Synthetic cryolite	8	42	79.0
Synthetic cryolite	6	59	70.5
Nicotine bentonite	8	174	13.0
Untreated check	0	200	—

Results of 1940 Experiments

In 1940, early, late, and combined early and late lead arsenate sprays at various levels were again tested. Each treatment, as well as an untreated check, was randomized in plots of one thirty-fifth of an acre replicated 10 times. A 15-gallon wheelbarrow sprayer was again used. The effectiveness of the sprays was measured by counting hornworm larvae on 10 per cent of the plants on August 7, 14, and 22, respectively, and also by recording, on August 23, all leaves moderately or severely injured; data for both criteria are transformed in Table 4 into percentages. Severely injured leaves were entirely worthless; moderately injured leaves were very greatly reduced in value; exact figures are not available.

TABLE 4.—THE EFFECT OF "EARLY" (AUGUST 2) AND "LATE" (AUGUST 9) APPLICATIONS OF LEAD ARSENATE SPRAYS AT DIFFERENT STRENGTHS ON THE HORNWORM POPULATIONS AND LEAF INJURY ON TOBACCO PLOTS AT VARYING PERIODS AFTER TREATMENT AT THE DOMINION EXPERIMENTAL SUBSTATION, DELHI, ONTARIO, AUGUST 2 TO 23, 1940

Treatment and rate per acre	Percentage control over check			Percentage of leaves injured as of August 23	
	First count August 7	Second count August 14	Third count August 22	Moderately	Severely
Early, 12 lb. Late, 12 lb.	89	97	95	1	0
Early, 12 lb. Late, 5 lb.	84	90	93	2	1
Early, 12 lb. Late, 12 lb.	89	87	88	2	1
Early, 5 lb. Late, 1 lb.	71	92	88	2	1
Early 5 lb. Late, 12 lb.	78	92	98	1	1
Late, 5 lb.	Not sprayed	73	79	9	7
Early, 5 lb.	58	72	56	6	4
No treatment	Nil	Nil	Nil	17	31

Table 4 indicates that with one exception treatments including early applications of lead arsenate resulted in excellent control of the larvae and hence less injury to the leaves. The exception, where 5 pounds of lead arsenate per acre were applied early, was apparently the result of an inadequate amount of poison. The improvement in control observed in the second larval count in this treatment may have occurred because the larvae had more time to ingest lethal amounts of lead arsenate.

Table 4 shows that a "late" spray of 5 pounds per acre apparently adequately replaced the lost residue of the "early" spray. If one bears in mind economy, reduction of poisonous residue on the leaves, and reduction of leaf injury these results indicate that the two treatments, 12 pounds "early", and 5 pounds "early" and 5 pounds "late", were the most practical of the series.

In 1940, an experiment was conducted to determine at what rate a single application of synthetic cryolite would achieve as good control as one treatment of lead arsenate at the rate of 6 pounds (in 40 gallons of water) per acre. A horse-drawn traction sprayer was used to gain experience in the control obtained with field equipment compared with previous work in which a hand-operated sprayer was employed.

The experimental area was 66 rows wide and 378 feet long. A total of 112 plots, 39 feet long and 4 rows wide, was laid out. The experiment involved 7 randomized treatments, replicated 16 times; these treatments were cryolite at 5 rates, lead arsenate at 6 pounds per acre, and no treatment as shown in Table 5.

TABLE 5.—A COMPARISON OF A SINGLE LEAD ARSENATE SPRAY AT 6 POUNDS PER ACRE WITH SYNTHETIC CRYOLITE SPRAYS AT 6 TO 36 POUNDS PER ACRE FOR THE CONTROL OF HORNWORMS AND LEAF INJURY ON TOBACCO AT THE DOMINION EXPERIMENTAL SUBSTATION, DELHI, ONTARIO, AUGUST 5 TO 23, 1940

Treatment and rate per acre; sprayed August 5	Percentage control over check		Percentage of leaves injured as of August 23	
	First count August 10	Second count August 23	Moderately	Severely
Cryolite, 36 lb.	82	81	2	1
Cryolite, 24 lb.	79	79	3	1
Arsenate of lead, 6 lb.	77	73	3	2
Cryolite 18 lb.	69	56	2	1
Cryolite, 12 lb.	67	58	3	2
Cryolite, 6 lb.	37	54	4	
No treatment	Nil	Nil	8	6

In Table 5, it will be noted that cryolite gave fair control of hornworms and good protection of the leaves when used at 18 pounds or more per acre; lower rates were not considered satisfactory. The results with 6 pounds of lead arsenate, under the conditions of the experiment, were only fair.

A third experiment was undertaken in 1940 to compare lead arsenate and synthetic cryolite, each applied as sprays and dusts of different strengths. Randomized treatments were applied on August 8 to two

TABLE 6.—A COMPARISON OF SYNTHETIC CRYOLITE AND LEAD ARSENATE SPRAYS AND DUSTS FOR HORNWORM CONTROL. NORWOOD FARM, LYNEDOCK, ONTARIO, AUGUST 8 TO 23, 1940

Treatment and rate per acre; applied August 8	Percentage control over check		Percentage of leaves injured	
	August 13	August 23	Moderately	Severely

Method—horse-drawn hand sprayer

Synthetic cryolite, 22 lb.	94	91	7	0
Lead arsenate, 2.5 lb.	82	89	5	1
Lead arsenate, 4.5 lb.	72	97	5	1
Synthetic cryolite, 5.5 lb.	79	86	4	1
Synthetic cryolite, 11 lb.	87	74	5	3

Method—horse-drawn power duster

Lead arsenate, 17 lb.	93	91	4	1
Synthetic cryolite, 16.5 lb.	93	89	3	1
Synthetic cryolite, 29. lb.	99	74	7	3
Lead arsenate 1, hydrated lime 8; 41.6* lb.	70	74	4	1
Cryolite, .1 lb.	63	34	13	6
Cryolite 1, celite †; 35.8† lb.	36	—6**	2	13
No treatment	Nil	Nil	15	14

* Five pounds only of this amount were arsenate of lead.

† Nine lb. only of this amount were cryolite.

** A negative sign preceding the percentage control figure indicates that the larval population exceeded that in the check plots.

blocks, each containing 12 plots. Those subjected to spraying were 4 rows wide and one-ninth of an acre in area, and the dusted plots were 6 rows wide and one-sixth of an acre in area. A horse-drawn hand-pumped sprayer and a horse-drawn power duster were used to apply the materials. The sprays were applied at the rate of approximately 40 gallons per acre.

Table 6 shows that a dust of lead arsenate at the rate of 17 pounds per acre gave very nearly as good results as the most effective spray. It is difficult to explain why sprays of lead arsenate at the rate of 4.5 pounds per acre, synthetic cryolite at the rate of 11 pounds per acre, and the dust of synthetic cryolite at the rate of 29.8 pounds per acre made such a relatively poor showing.

Synthetic cryolite dusts generally did not remain effective for so long a period as did lead arsenate dusts or the liquid sprays of either poison. The percentage reduction of larvae fell from 63 to 34 and from 99 to 74, respectively within 9 days when cryolite dusts were used at 6.1 and 29.8 pounds per acre. For some reason this loss of effectiveness was not nearly so marked when 16.5 pounds of cryolite were used. It will also be seen that sprays of both materials and dusts of lead arsenate in general either increased the percentage of control or dropped only slightly in effectiveness during the period of observation.

Results of 1947 Experiments

No further insecticidal tests were conducted on tobacco in the field until 1947, when sprays of the newer insecticides DDT, chlordane, benzene hexachloride, and ryania were compared with those of lead arsenate.

Thirty plots of one-third of an acre each were laid out on the de Meyere farm near Thamesville, Ontario. These plots were subjected to 10 randomized spray treatments, each replicated 3 times. The treatments were applied on August 8, at the rate of approximately 40 gallons per acre by means of a horse-drawn, manually operated 4-row sprayer with a capacity of 40 gallons.

TABLE 7.—A COMPARISON OF SPRAYS OF DDT, LEAD ARSENATE, RYANIA, CHLORDANE, AND BHC AT DIFFERENT STRENGTHS FOR THE CONTROL OF HORNWORMS ON TOBACCO AT THE DE MEYERE FARM, THAMESVILLE, ONTARIO, AUGUST 8 TO 15, 1947

Treatment applied August 8	Pounds of poison per acre	Number of larvae per treatment		Percentage control over check	
		August 12	August 15	August 12	August 15
DDT	1.3	2	1	99.5	99.7
DDT	2.2	6	3	98.6	99.1
DDT	0.72	15	4	96.4	98.8
Lead arsenate	4.7	28	13	93.4	95.9
Lead arsenate	2.2	119	47	72.1	95.1
Ryania	2.5	80	66	81.2	79.0
Chlordane	1.5	131	29	69.3	90.8
Chlordane	0.65	240	212	43.8	32.8
BHC	0.18*	436	294	-2.1	6.7
Untreated check	Nil	427	314	—	—

* This amount contained 6 per cent gamma isomer.

Data in Table 7 show that DDT applied as a 50 per cent wettable powder in water was the best insecticide at any of the rates tried. Applications of 2.2, 1.3, and 0.72 pounds of actual DDT per acre, all gave excellent control. The results with chlordane were variable but the 1.5 pound rate gave fair control. Ryania showed some promise but, in this instance, was slightly below "commercial control". Benzene hexachloride, at the strength applied, was disappointing. It should be noted that lead arsenate continued to give good commercial control and was second only to DDT in effectiveness.

Results of 1948 Experiment

In 1948, an experiment was conducted at the Dominion Experimental Substation, Delhi, Ontario, to determine if a single application of DDT dust was practical and adequate for the control of the hornworm on tobacco. Plots of one-tenth of an acre were dusted on July 22 with the following preparations:

- 5 per cent DDT,
- 3 per cent DDT,
- 3.2 per cent lead arsenate.

These preparations were applied on July 22 at the rate of 30 pounds per acre by means of a motor-driven wheelbarrow duster. Each treatment was replicated 3 times in a random design.

Larval counts were taken on July 27, August 4, August 11, and August 27, i.e. 5, 13, 20, and 26 days after treatment. Each count was based on 200 plants or nearly 40 per cent of the total. These were selected at random in each plot and examined for larvae. Outside rows and the ends of rows were avoided to prevent interference from adjacent treatments.

Table 8 shows that both 5 per cent and 3 per cent dusts of DDT gave commercially adequate controls of 72.5 per cent and 70.5 per cent, respectively. The lead arsenate dust was worthless in this experiment.

TABLE 8.—THE RESULTS OF EXPERIMENTAL TRIALS WITH DDT AND LEAD ARSENATE DUSTS FOR HORNWORM CONTROL ON TOBACCO PLOTS AT THE DOMINION EXPERIMENTAL SUBSTATION, DELHI, ONTARIO, JULY 22 TO AUGUST 27, 1948

Treatment applied July 22	Number of larvae per treatment				Percentage control over check				Seasonal average per cent control
	July 27	Aug. 4	Aug. 11	Aug. 27	July 27	Aug. 4	Aug. 11	Aug. 27	
DDT, 5 per cent	6	1	36	31	66.7	92.9	67.6	63.1	72.5
DDT, 3 per cent	10	0	48	16	44.4	100.0	56.8	80.9	70.5
Lead arsenate, 3.2 per cent	17	1	139	97	5.6	21.5	-75.2	-15.4	-3.3
Untreated check	18	14	111	84	0	0	0	0	—

DISCUSSION

Study of these tables indicates that where arsenate of lead was used a thoroughly applied early spray was as effective as two sprays applied early and late, provided the total amount of poison applied per acre was com-

parable. The large amount of leaf injury suffered in plots receiving a single "late" treatment ruled out the advisability of relying on late application for adequate control.

As a result of these experiments, it is now recommended that where synthetic cryolite is used it should be applied at a rate of 18 to 20 pounds per acre. Because of the higher cost of cryolite, it has not been widely employed by growers.

Of all the insecticides tested, DDT was by far the most effective, even at the comparatively low concentration of 0.72 pounds per acre; moreover, DDT does not leave so much undesirable residue on the foliage as lead arsenate or cryolite.

In these experiments, sprays were generally more effective than dusts, but experienced tobacco growers who have used DDT dusts have expressed complete satisfaction with the results.

No injury which could be attributed to the insecticides was seen on the tobacco leaves in any of the experiments.

In conclusion, it is felt that DDT, as a control for hornworm, fulfils many of the requirements of an ideal insecticide and that it has solved, for the time being at least, the problem of finding an acceptable insecticide to control the tomato hornworm on tobacco.

SUMMARY

A brief history of the tomato hornworm on tobacco and its control in Ontario is given.

Field experiments on the control of the tomato hornworm on tobacco by means of chemicals, conducted during 1938, 1939, 1940, 1947, and 1948 by the staff of the Dominion Entomological Laboratory at Chatham, Ontario, are outlined. All tests were run on flue-cured tobacco, chiefly at the Dominion Experimental Substation, Delhi, Ontario.

The insecticides tested included ryania, nicotine bentonite, synthetic cryolite, barium fluosilicate, calcium arsenate, lead arsenate, chlordane, benzene hexachloride, and DDT. Of these, DDT was by far the most effective, even at concentrations as low as 0.72 pounds of actual DDT per acre, and the prediction is made that this material will probably replace other poisons now in use in Ontario.

ACKNOWLEDGMENT

Acknowledgment and thanks are extended to the following members and former members of the staff of the Dominion Entomological Laboratory, Chatham, Ontario, who did much of the work involved in the experiments reported: Messrs. G. Beall, G. E. Coppel, D. S. Marshall, J. L. Whitlock, A. A. Wood; and to Messrs. F. A. Stinson and L. Vickery of the Dominion Experimental Substation, Delhi, Ontario, for assistance and facilities provided.

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SOIL MOISTURE STUDIES

IV. INDIRECT DETERMINATION OF FIELD CAPACITY FOR MOISTURE¹

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In a previous paper in this series (9), it was suggested that the field capacity of the soil for moisture could be used as a basis for supplemental classification of soils following a soil survey. This method has subsequently been used (10, 11, 12) to good effect in classifying soils with respect to moisture-holding capacity (as representing soil texture), and in correlating field capacity for moisture with other factors. In this subsequent work, the field capacity was determined by laboratory procedures, as outlined in the second paper of this series (8).

When the investigation noted above was undertaken, soil samples were collected 24 hours after irrigating, at a standard depth of 8 to 12 inches. These samples contained very little organic matter. The question arose, therefore, as to whether the results obtained could safely be applied to A horizons, containing organic matter in moderate amount. In an attempt to answer this question, it was decided to repeat the investigation, obtaining the samples from two depths representing different contents of organic matter. It was anticipated that the curves and equations obtained would also serve to check the results already reported (8). This present paper summarizes the results of the second investigation.

In view of the fact that the literature on this subject was covered in the second paper of this series (8), it will not be repeated in this paper.

PROCEDURE

A total of 54 locations were selected in irrigated orchards in the Okanagan Valley, in 1944 and 1945. A wide range of soil texture was represented. Soil samples were taken approximately 24 hours after completion of an irrigation. At each location, two samples were taken at a depth of 4 to 8 inches, and two at a depth of 10 to 14 inches. Each sample was taken with a sampling can 3 inches in diameter, 4 inches deep, and of known volume. The two samples from each depth were composited. Where gravel or stones were encountered, the samples were discarded; thus only one depth was represented at some locations.

In the laboratory, each composited soil sample was weighed, screened through a 3 mm. sieve, mixed thoroughly, and dried. Calculations were made on volume weight, field capacity for moisture in percentage dry weight, and field capacity in inches of water per foot of soil.

The wilting coefficients of most of the samples were determined by growing sunflowers in a portion of each sample in the greenhouse. The available moisture contents were determined by deducting the wilting coefficient figures from the field capacity figures.

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The moisture equivalents were determined with an International clinical centrifuge, using a modification of the procedure suggested by Goldbeck and Jackson (3). The centrifuge was run for 30 minutes at a speed sufficient to produce a centrifugal force of 200 atmospheres, which gave results very close to those obtained in a standard moisture equivalent centrifuge at 1000 atmospheres. This apparent discrepancy can be attributed primarily to differences in packing of the soil and to the use of a different type of centrifuge head.

Other measurements made on the soil samples included the settling volume, mechanical analysis, and organic matter content. The settling volume was determined by the procedure described by Wilcox and Spilsbury (8), the mechanical analysis by the Bouyoucos hydrometer procedure (1), and the organic matter content by the hydrogen peroxide method of Robinson (5) as modified by Wilcox and Walker (12).

Correlations were determined between field data and laboratory measurements, and between different types of laboratory determinations.

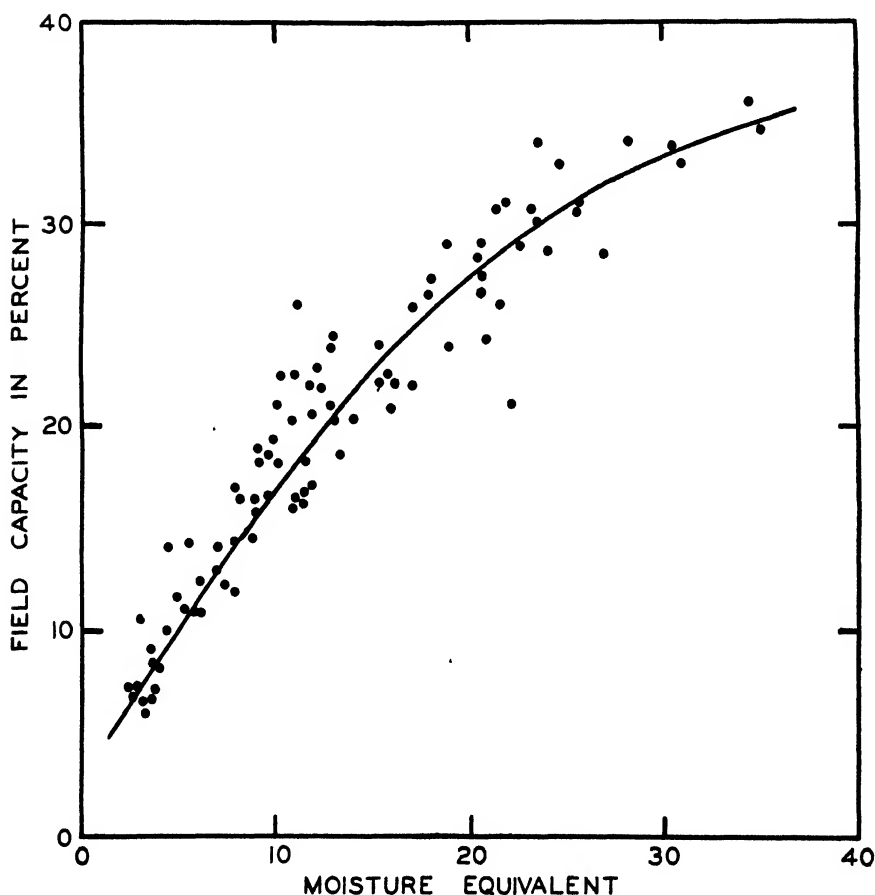


FIGURE 1. Scatter diagram of field capacity in per cent charted against moisture equivalent. The curve shown has the equation $y = 2.29 + 1.66x - 0.0207x^2$, in which y = field capacity and x = moisture equivalent.

Both straight line equations and second degree polynomial equations (6) were calculated for determining the field capacity, wilting coefficient and available moisture content from the various laboratory measurements. Since previous work (8) had indicated that correlations with silt content gave low and unreliable coefficients, no calculations involving silt content were made. The standard error was determined for each equation, and was expressed in percentage of the mean of the function being determined.

RESULTS

The more pertinent data obtained in the course of this investigation are summarized in Table 4. Figures for available moisture have been omitted from the table, but these can readily be calculated by deducting the wilting coefficient figures from the field capacity figures.

Effects of Depth of Sampling

It was anticipated that the higher organic matter contents in the 6-inch (i.e. 4-8 inch) samples would be accompanied by higher field capacities, in comparison with the 12-inch (i.e. 10-14 inch) samples. However, this did not prove to be true. More often than not, the field capacity was higher in the 12-inch samples than in the 6-inch samples. Typical results are presented in Table 1.

TABLE 1.—COMPARISON OF 6-INCH AND 12-INCH SAMPLES

Sample No.	Average depth	Organic matter	Field capacity	Moisture equivalent	Colloid
	in	%	%	%	%
2a	6	0.75	11.8	7.8	11.3
2b	12	0.50	16.6	9.6	13.9
6a	6	0.77	21.9	12.4	18.9
6b	12	0.34	22.8	12.2	17.4
19a	6	1.50	34.0	23.6	44.6
19b	12	0.67	32.8	24.6	45.2
23a	6	1.13	20.8	16.0	38.1
23b	12	0.70	28.5	26.8	57.5
34a	6	0.27	6.6	3.4	6.9
34b	12	0.03	7.3	3.0	8.9
35a	6	0.24	9.1	3.6	8.4
35b	12	0.02	7.3	2.8	6.8
53a	6	0.34	33.8	30.4	76.6
53b	12	0.02	34.7	35.0	83.8

In spite of higher contents of organic matter in the 6-inch samples, the colloid content was usually higher in the 12-inch samples. The reason for this has not been ascertained. It is suspected to be due to movement of the finer soil particles from the surface soil downward into the subsoil, especially in the sandy soils. The moisture equivalent showed a close correlation with both the field capacity and the colloid content, as indicated in Table 1. It might be noted that the organic matter contents were all comparatively low.

TABLE 2.—COEFFICIENTS OF CORRELATION

	Percentage sand	Percentage clay	Percentage colloid	Settling volume	Moisture equivalent
Field capacity, in per cent	-0.92	+0.82	+0.86	+0.91	+0.93
Wilting coefficient, in per cent	-0.88	+0.85	+0.91	+0.94	+0.94
Available moisture, in per cent	-0.91	+0.76	+0.82	+0.89	+0.89
Field capacity, in inches	-0.89	+0.76	+0.79	+0.88	+0.89
Wilting coefficient, in inches	-0.92	+0.82	+0.87	+0.92	+0.92
Available moisture, in inches	-0.85	+0.70	+0.72	+0.82	+0.83
Volume weight	+0.85	-0.80	-0.81	-0.86	-0.87
Settling volume	-0.95	+0.89	+0.94	—	+0.88
Moisture equivalent	-0.94	+0.93	+0.96	+0.88	—

These results give no indication of any distinct effect of the organic matter content on the relations between field capacity on the one hand and moisture equivalent or colloid content on the other hand; at least, at the low organic matter contents encountered in this investigation. Accordingly, all of the soil samples were grouped together in calculating the correlations and equations reported below.

Correlations

Those correlations of special interest are presented in Table 2. They are all "highly significant", with odds greater than 99 : 1. On the whole, the coefficients listed are similar in value to those reported previously (8). A new feature is the column of correlations with moisture equivalent. These give coefficients almost the same as for those with settling volume. It will be noted that the correlations involving laboratory measurements only (such as between moisture equivalent and percentage colloid) tend to have distinctly higher coefficients than those including one field measurement (such as available moisture in inches and percentage colloid).

Equations

One of the major purposes of this investigation was to assess the accuracy of different laboratory methods of determining field capacity. To accomplish this, it was necessary to calculate the equations that could be used for determining field capacity from each of the laboratory determinations. This was done in two ways:

(a) on the basis of the equation

$$y = a + bx,$$

(b) on the basis of the equation

$$y = a + bx + cx^2$$

In these equations,

y = the function (e.g. field capacity in per cent).

x = the factor (e.g. moisture equivalent).

a, b, c = constants.

The first equation assumes a straight-line trend between the two values, the second a curved trend of the second-degree polynomial type. In a previous investigation (8), it was found that nothing was to be gained by using more than one factor in each equation. In this investigation, accordingly, only one factor was used at a time in making the calculations.

In addition to field capacity, equations have also been calculated involving wilting coefficient and available moisture. In almost every case, the curve equations were found to fit the distributions more accurately than the straight-line equations, and to give lower standard errors of estimate. In comparing the laboratory methods, therefore, it will be considered sufficient to do so on the basis of standard errors using the curve equations only. Such a comparison is presented in Table 3.

In three cases, the errors involved in using the straight-line equations were about the same as those from the curves. These were as follows:

- Wilting coefficient in per cent, from moisture equivalent (18.5 per cent error)
- Available moisture in per cent, from settling volume (17.3 per cent error)
- Wilting coefficient in inches, from settling volume (17.1 per cent error).

The selection of a laboratory procedure to use in estimating field capacity, wilting coefficient or available moisture will depend not only on the percentage error involved, but also on the type of curve obtained and the ease of making the laboratory determinations. The closer the curve is to a straight line, the more readily can it be used for transposing values

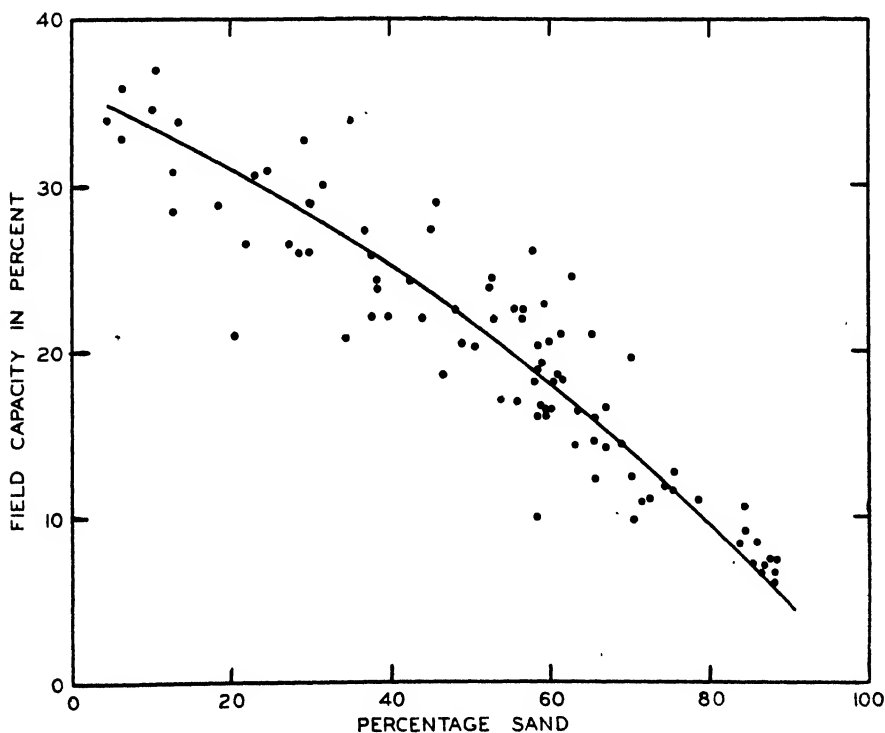


FIGURE 2. Scatter diagram of field capacity in per cent charted against percentage sand. The curve shown has the equation $y = 35.76 - 0.200x - 0.00158x^2$, in which y = field capacity and x = percentage sand.

TABLE 3.—STANDARD ERRORS OF ESTIMATE, IN PERCENTAGE OF THE MEAN OF THE FUNCTION

Function being determined	Factor used in determination				
	Percentage sand	Percentage clay	Percentage colloid	Settling volume	Moisture equivalent
Field capacity, in per cent	9.7	17.4	12.7	13.3	10.9
Wilting coefficient, in per cent	22.6	22.8	19.6	20.6	18.7
Available moisture, in per cent	13.3	20.3	17.4	17.5	11.8
Field capacity, in inches	10.6	16.6	14.0	15.0	13.3
Wilting coefficient, in inches	15.9	18.2	16.6	16.2	15.3
Available moisture, in inches	12.5	19.6	16.9	18.2	14.2

throughout the range encountered. Taking both percentage error and type of curve into account, the most satisfactory of the equations for routine use can be listed in order of preference as follows:

Field capacity in per cent:

- (1) From moisture equivalent, $y = 2.29 + 1.66x - 0.0207x^2$. See Figure 1.
- (2) From percentage sand, $y = 35.76 - 0.200x - 0.00158x^2$. See Figure 2, and compare with Figure 3.
- (3) From settling volume, $y = -41.95 + 1.89x - 0.01019x^2$.

Wilting coefficient in per cent:

- (1) From moisture equivalent, $y = 0.92 + 0.317x$. See Figure 4.
- (2) From percentage colloid, $y = 0.82 + 0.186x - 0.000762x^2$.
- (3) From settling volume, $y = -10.97 + 0.399x - 0.000865x^2$.

Available moisture in per cent:

- (1) From moisture equivalent, $y = 0.89 + 1.494x - 0.0244x^2$. See Figure 5.
- (2) From percentage sand, $y = 25.74 - 0.131x - 0.00127x^2$.

Field capacity in inches per foot of soil:

- (1) From percentage sand, $y = 5.326 - 0.0205x - 0.000313x^2$.
- (2) From moisture equivalent, $y = 1.12 + 0.23x - 0.00329x^2$.
- (3) From percentage colloid, $y = 1.04 + 0.120x - 0.00090x^2$.

Wilting coefficient in inches per foot of soil:

- (1) From moisture equivalent, $y = 0.13 + 0.0654x - 0.000519x^2$.
- (2) From percentage sand, $y = 1.635 - 0.0117x - 0.0000539x^2$.

Available moisture in inches per foot of soil:

- (1) From percentage sand, $y = 3.85 - 0.0138x - 0.000207x^2$.
- (2) From moisture equivalent, $y = 0.13 + 0.279x - 0.00572x^2$.

In each of these equations, y represents the function and x represents the factor as noted above. The first equation, for example, could have been written as follows:

$$\text{Field capacity in per cent} = 2.29 + 1.66 (\text{moisture equivalent}) - 0.0207 (\text{moisture equivalent})^2.$$

The moisture equivalent appears, from this investigation, to be preferable to either the settling volume or mechanical analysis as an indirect method of determining the field capacity. Where a mechanical analysis has already been made, however—such, for example, as in the course of a soil survey—the data obtained can readily be used for estimating the field capacity.

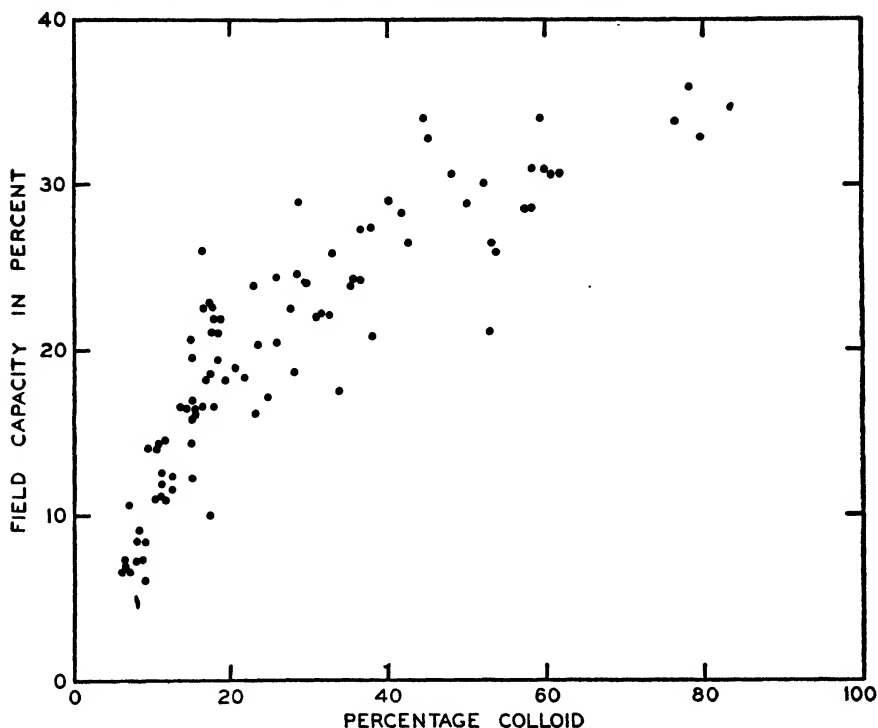


FIGURE 3. Scatter diagram of field capacity in per cent charted against percentage colloid.

A useful equation is that calculated for use in determining the field capacity in inches of water per foot of soil from the field capacity in per cent:

$$y = -0.040 + 0.214x - 0.00197x^2.$$

The use of this equation obviates the necessity of determining the volume weight of the soil in situ. The standard error of estimate was only 6.2 per cent of the mean, which is quite low for equations based on field determinations.

Relation Between Soil Texture and Available Moisture

Soil texture has been measured directly by mechanical analysis, and indirectly by settling volume and moisture equivalent determinations. The figures obtained from the mechanical analysis for percentages of sand, silt, clay and colloid all contribute to our knowledge of the texture of the soil. No one of these figures, however, gives the full story by itself.

It can be seen from the correlations noted above, that no matter how the texture was measured, there were strong tendencies for both the field capacity and the wilting coefficient to increase as the soil particles became finer. The question arises as to whether the difference between the field capacity and the wilting coefficient (i.e. the available moisture) should increase in similar manner. The strong positive correlations between available moisture and those measurements representing texture indicate

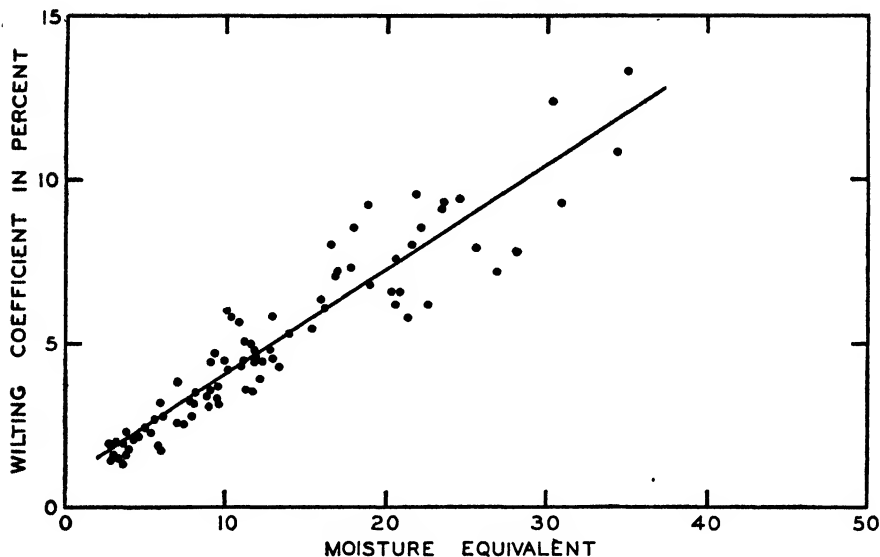


FIGURE 4. Scatter diagram of wilting coefficient in per cent charted against moisture equivalent. The curve shown has the equation $y = 0.92 + 0.317x$, in which y = wilting coefficient and x = moisture equivalent.

that it does. An examination of the various charts, however, reveals a general tendency for the available moisture to increase to a maximum and then decrease as the soil becomes still heavier. From the equations for the curves, the available moisture in per cent was found to be at a maximum at the following values:

- Maximum of 25.7 when per cent sand = 0.
- Maximum of 23.6 when per cent clay = 48.9.
- Maximum of 23.4 when per cent colloid = 69.3.
- Maximum of 23.7 when moisture equivalent = 30.0.

The available moisture in inches was at a maximum at the following values:

- Maximum of 3.85 when per cent sand = 0.
- Maximum of 3.43 when per cent clay = 35.2.
- Maximum of 3.40 when per cent colloid = 51.3.
- Maximum of 3.54 when moisture equivalent = 24.4.

Judging by the data from per cent clay, per cent colloid and moisture equivalent, the available moisture content of the soil does not increase indefinitely as the soil particles decrease in size. The maximum available moisture in per cent was at a clay content of about 50 per cent or a colloid content of about 70 per cent; and the maximum available moisture in inches of water was at a clay content of about 35 per cent or a colloid content of about 50 per cent. Of these four figures, the latter two are more applicable to field conditions, as the actual quantity of water available for plant use is measured in volume rather than in per cent. According to this, then, very heavy soils contain no higher content of available moisture than do moderately heavy soils with a clay content of about 35 per cent. This confirms a similar finding reported in 1941 (8).

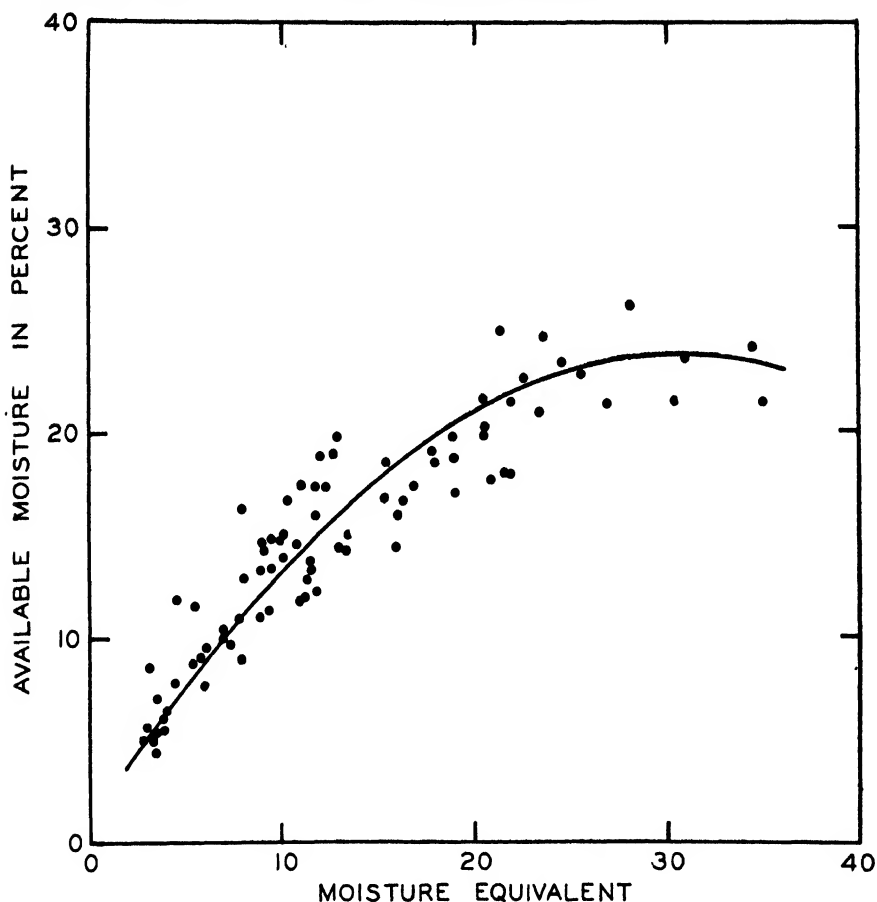


FIGURE 5. Scatter diagram of available moisture in per cent charted against moisture equivalent. The curve shown has the equation $y = 0.89 + 1.494x - 0.0244x^2$, in which y = available moisture and x = moisture equivalent.

The curves of available moisture plotted against per cent sand did not conform to those plotted against the other laboratory measurements. The available moisture content increased as the per cent sand decreased, right down to a sand content of zero. This was true whether the available moisture was expressed in per cent or in inches. This discrepancy between the sand curves on the one hand and the clay and colloid curves on the other hand appears to be due to two causes: *first*, the unpredictable effects of the silt content; and *second*, the fact that when the sand content approached zero, the soil could often be considered only "moderately heavy". A soil containing 35 per cent clay, 60 per cent silt and 5 per cent sand, i.e. a "silty clay soil", would be considered rather heavy; but it could be much heavier still with very little difference in the sand content.

Comparison of Field Data and Laboratory Data

In order to study laboratory methods of determining the moisture-holding capacity of the soil under field conditions, it has been considered necessary to use samples actually collected in the field at or near the

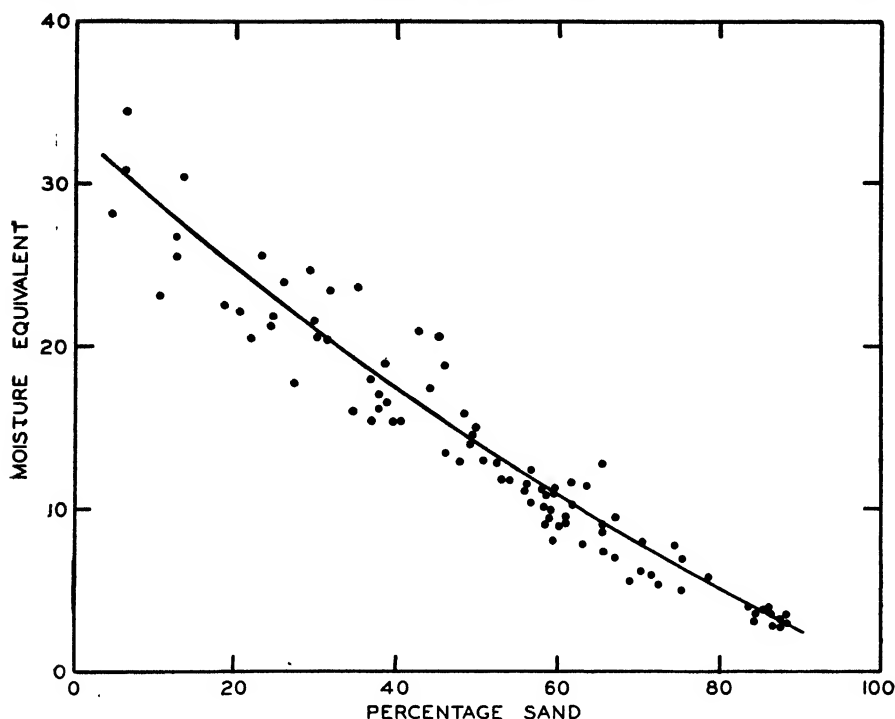


FIGURE 6. Scatter diagram of moisture equivalent charted against percentage sand. The curve shown has the equation $y = 33.35 - 0.447x + 0.00154x^2$, in which y = moisture equivalent and x = percentage sand.

moisture-holding capacity. It is recognized that the moisture content in the field is affected by many factors not operative in the laboratory, and is therefore more variable. The fact that field conditions are not simulated in the laboratory, however, constitutes a valid reason for obtaining the basic data right in the field.

The question arises as to what extent factors other than texture that are operative in the field affect the field capacity. In this present investigation, the best field and laboratory data available for comparison are the field capacity and the moisture equivalent. An examination of Table 2 reveals the following comparisons of coefficients of correlation:

Between field capacity and per cent sand	- 0.92
Between moisture equivalent and per cent sand	- 0.94
Between field capacity and per cent clay	+ 0.82
Between moisture equivalent and per cent clay	+ 0.93
Between field capacity and per cent colloid	+ 0.86
Between moisture equivalent and per cent colloid	+ 0.96

The correlations with field capacity were surprisingly high. On the whole, however, the correlations with the moisture equivalent were higher still.

The value of a coefficient of correlation depends primarily on two factors—*first*, the direction of the line of trend; and *second*, the distribution of the values away from this line. An examination of the scatter diagrams indicates that in every case the points were more widely scattered in the

field capacity charts than in the moisture equivalent charts. By way of example, Figure 2 can be compared with Figure 6, and Figure 3 with Figure 7.

Some of the sources of variability encountered in determining the field capacity are as follows:

(1) Variations in time that it takes the excess water to drain into the subsoil. It usually takes much longer with a heavy soil than a light soil. Choice of 24 hours after irrigation does not allow complete drainage in many cases before sampling.

(2) Variations in rate of absorption of moisture by plant roots during the 24 hours.

(3) Variations in the subsoil below. As already reported (7) more water remains above either a clay pan or a gravel layer than above a uniform silt or fine sand.

Under field conditions, it is impossible to select any one time after wetting when soils of varying type and under varying cultural treatment can all be said to be at the moisture-holding capacity. The use of 24 hours is merely a compromise between rate of drainage and rate of root absorption. At greater depths than those used in this investigation (6 inches and 12 inches), it would appear advisable to increase the interval between irrigating and sampling to more than 24 hours.

Because of the effects of these various factors on the moisture-holding capacity under field conditions, there is a wide variation from location to location in the field capacity of soil samples apparently almost identical in textual characteristics. This raises considerably the calculated standard error of conversion from a laboratory determination to the field capacity. As noted in Table 3, the standard errors determined in this investigation were comparatively high. Such a situation is unavoidable.

It still holds true that the best method of determining the true field capacity at any location is to take samples periodically in the field following an irrigation. Not only is there a source of error in making the conversion from a laboratory determination to the field capacity, but no laboratory method can take into account the effects of the various factors encountered in the field. To determine accurately the field capacity at any one location, it is necessary to determine it right at that location.

If absolute accuracy in determining the field capacity for moisture is not considered essential, however, then the use of a laboratory procedure may not only be permissible but advisable. Of those methods tested in this investigation, the moisture equivalent appears to be the most reliable. Other investigators (2, 4) have already used the moisture equivalent for this purpose, and have expressed or converted the values obtained by means of curves.

The question has frequently arisen as to whether the moisture equivalent can safely be used to represent the field capacity without the use of a converting factor or equation. An examination of Figure 1 will indicate that this would be risky, as the line of trend between the two is a definite curve. It will also be noted that the curves obtained from per cent sand and per cent colloid charted against field capacity (Figures 2 and 3)

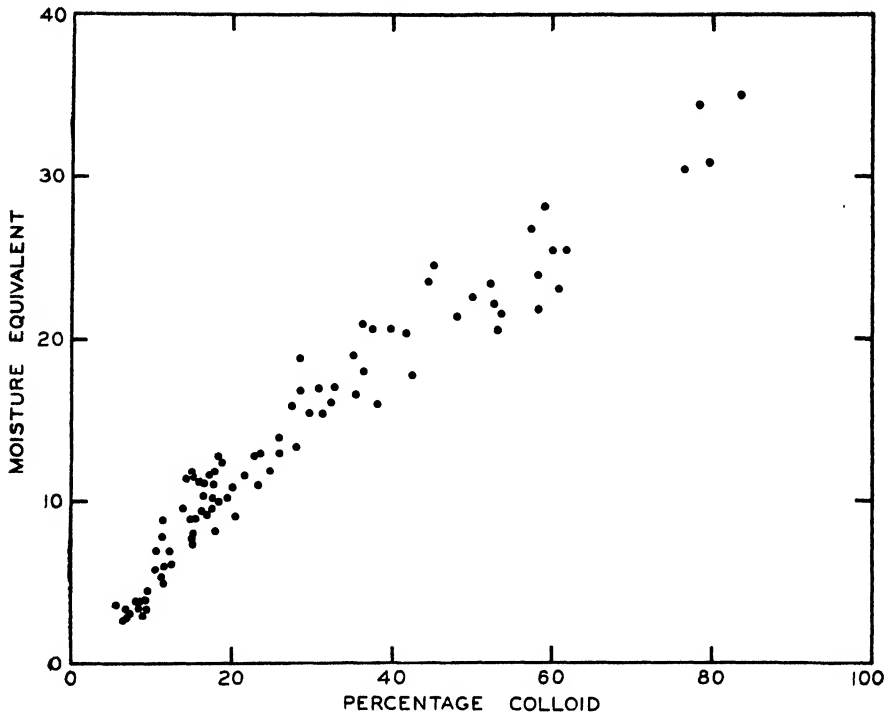


FIGURE 7. Scatter diagram of moisture equivalent charted against percentage colloid.

are somewhat different in shape from those of per cent sand and per cent colloid charted against moisture equivalent (Figures 6 and 7). If the moisture equivalent is to be used for estimating the field capacity, it appears safest to use the curve or its equation for transposing the values. It should be noted that the moisture equivalent was not determined in this investigation by the standard procedure, and that the curves obtained could not safely be used for standard moisture equivalent values without checking them first.

SUMMARY

Soil samples were collected from Okanagan orchards approximately 24 hours after an irrigation, at depths of 4-8 and 10-14 inches. A total of 93 samples was obtained, of known volume. The field capacity of each was determined, expressed both in per cent by weight and in inches of water per foot of soil. Greenhouse determinations were made of the wilting coefficient. The "available" moisture was determined by deducting the wilting coefficient from the field capacity. Laboratory determinations were made of the moisture equivalent, the settling volume, the mechanical analysis, and the organic matter content.

No effect of organic matter content was apparent on the field capacity at each location. In most cases, the 4-8 inch sample had a higher organic matter content, a lower colloid content, and a lower field capacity than the 10-14 inch sample.

High coefficients of correlation were obtained between the laboratory determinations on the one hand and field capacity, wilting coefficient and available moisture on the other hand. Still higher coefficients were obtained among the laboratory determinations.

Second degree polynomial equations were calculated for determining field capacity, wilting coefficient and available moisture from each of the laboratory determinations. The standard errors of estimate were high, ranging mostly between 10 and 20 per cent of the means. The best all-round laboratory measurement for use in indirect determination of the three moisture measurements proved to be the moisture equivalent. However, the percentage sand and percentage colloid also proved reasonably satisfactory.

Evidence is presented to indicate that as the soil particles become finer, the moisture content available for plant use increases to a certain point only. The maximum content of available moisture was obtained at a clay content of around 35 per cent.

[*Appendix (Table 4) on pages 576-578*]

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APPENDIX

TABLE 4.—SUMMARY OF SOILS DATA

Sample No.	Sand	Silt	Clay	Colloid	Organic matter	Volume weight	Settling volume	Moisture equivalent	Field capacity*		Wilting coefficient*	
	%	%	%	%	%		cc.	%	%	in.	%	in.
1a**	63.4	28.0	8.6	14.5	—	1.52	40.0	11.4	16.4	2.99	3.6	0.66
2a	74.4	18.9	6.7	11.3	0.75	1.55	37.0	7.8	11.8	2.21	2.8	0.52
2b	67.0	26.1	6.9	13.9	0.50	1.58	37.0	9.6	16.6	3.15	3.2	0.61
3a	63.1	27.5	9.4	15.0	—	1.47	38.0	7.8	14.4	2.57	3.5	0.62
3b	58.0	32.2	9.8	16.6	0.13	1.39	40.0	11.2	26.0	4.35	—	—
4b	70.4	18.6	11.0	15.1	0.03	1.51	36.0	8.0	19.5	3.34	3.2	0.57
5a	86.5	8.1	5.4	6.6	—	1.48	34.0	3.6	6.7	1.24	1.4	0.25
5b	85.4	7.7	6.9	8.1	—	1.55	33.0	3.8	7.2	1.34	1.7	0.32
6a	56.6	30.9	12.5	18.9	0.02	1.44	43.0	12.4	21.9	3.79	4.5	0.78
6b	59.4	31.0	9.6	17.4	0.77	1.48	41.0	12.2	22.8	4.06	3.9	0.69
7a	75.4	15.1	9.5	12.2	0.18	1.54	37.5	7.0	12.6	2.33	2.6	0.48
7b	69.0	23.7	7.3	10.9	—	1.50	37.0	5.6	14.3	2.58	2.7	0.49
8a	60.9	28.1	11.0	17.7	1.13	1.41	44.0	9.6	18.6	3.14	3.7	0.63
9a	45.1	26.1	28.8	37.8	—	1.37	50.0	20.6	27.4	4.50	7.6	1.25
10a	42.5	32.0	25.5	36.5	1.48	1.40	52.5	20.8	24.3	4.11	6.6	1.11
11a	56.6	31.4	12.0	16.5	1.30	1.43	42.0	10.4	22.5	3.85	5.8	1.00
12a	58.6	27.8	13.6	20.1	1.40	1.46	45.0	10.8	20.3	3.57	5.7	1.00
12b	61.5	28.2	10.3	17.8	—	1.49	41.5	10.2	21.1	3.78	6.0	1.07
13a	59.4	26.7	13.9	23.3	0.78	1.49	43.0	11.0	16.2	2.89	4.3	0.77
13b	60.2	29.3	10.5	15.6	0.29	1.40	40.5	9.0	16.4	2.76	3.1	0.52
14a	71.6	21.0	7.4	11.7	0.17	1.67	35.0	6.0	10.9	2.19	3.2	0.64
14b	86.9	8.5	4.6	6.8	—	1.67	30.5	2.8	7.0	1.40	2.0	0.40
15a	78.7	14.0	7.3	10.5	0.80	1.55	36.0	5.8	11.0	2.06	1.9	0.35
15b	75.3	16.9	7.8	11.5	0.24	1.59	34.5	5.0	11.6	2.22	2.4	0.46
16a	58.4	30.4	11.2	17.5	—	1.57	34.0	4.4	10.0	1.89	2.2	0.41
16b	85.9	7.7	6.4	8.2	—	1.48	35.0	3.8	8.4	1.49	2.3	0.41
17b	59.0	30.0	11.0	18.5	—	1.38	43.0	10.0	19.3	3.75	4.5	0.75
18a	58.2	30.1	11.7	19.3	—	1.42	39.5	10.2	18.2	3.12	4.2	0.72
19a	35.0	31.8	33.2	44.6	1.50	1.23	55.5	23.6	34.0	5.06	9.3	1.37
19b	29.2	39.2	31.6	45.2	0.67	1.29	52.5	24.6	32.8	5.10	9.4	1.46
20a	55.9	31.4	12.7	17.8	0.97	1.44	42.0	11.2	22.6	3.99	5.1	0.88

21a	49.0	34.4	16.6	26.0	1.00	1.35	48.0	14.0	20.4	3.31	5.3	0.86
21b	50.6	35.2	14.2	23.5	1.03	1.37	45.5	13.0	20.3	3.33	5.8	0.95
22a	46.6	39.2	14.2	28.1	0.78	1.46	47.0	13.4	18.6	3.25	4.3	0.75
22b	21.9	52.5	25.6	53.2	0.45	1.34	52.0	20.6	26.5	4.26	6.2	1.00
23a	34.5	47.3	18.2	38.1	1.13	1.37	52.0	16.0	20.8	3.42	6.3	1.04
23b	12.6	58.9	29.4	57.5	0.70	1.29	57.0	26.8	28.5	4.43	7.2	1.12
24a	37.6	46.7	17.7	32.6	1.00	1.3	50.0	16.2	22.1	3.55	6.1	0.97
24b	18.6	54.7	26.7	50.1	0.37	1.31	54.0	22.6	28.9	4.55	6.2	0.98
25a	48.2	32.3	19.5	27.7	1.30	1.46	47.0	15.8	22.5	4.03	—	—
25b	45.8	34.6	19.6	28.8	1.21	1.27	49.5	18.8	29.0	4.45	9.2	1.40
26a	72.4	21.0	6.6	11.2	0.67	1.67	33.5	5.4	11.1	2.22	2.3	0.46
26b	70.2	21.0	8.8	12.6	0.50	1.63	34.5	6.2	12.4	2.44	2.8	0.55
27a	10.6	39.8	49.6	60.8	1.98	1.29	58.5	23.2	30.7	4.78	—	—
28a	53.0	36.0	11.0	17.8	1.12	1.45	42.0	11.8	21.9	3.81	4.5	0.78
28b	65.4	20.4	14.2	18.4	0.63	1.47	42.0	12.8	21.0	3.70	—	—
29a	30.0	48.0	22.0	40.0	1.03	1.27	48.5	20.6	29.0	4.41	—	—
29b	31.2	45.2	23.6	41.8	0.72	1.35	50.0	20.4	28.3	4.60	6.6	1.07
30a	24.4	47.4	28.2	48.2	0.52	1.31	54.5	21.4	30.7	4.81	5.8	0.91
30b	4.6	60.2	35.2	59.2	0.29	1.25	55.5	28.2	34.0	5.12	7.8	1.17
31a	54.0	30.8	15.2	24.8	1.22	1.42	43.7	11.8	17.1	2.92	4.8	0.82
31b	47.8	36.6	15.6	26.0	0.85	1.34	45.5	13.0	24.4	3.92	4.6	0.74
32a	52.4	31.2	16.4	23.0	0.99	1.43	44.0	12.8	23.8	4.09	4.8	0.82
33a	55.2	38.2	6.6	9.6	0.20	1.59	34.0	4.6	14.1	2.68	2.2	0.42
33b	84.4	8.6	7.0	7.2	0.23	1.63	33.0	3.2	10.6	2.08	2.0	0.39
34a	88.2	6.4	5.4	6.9	0.27	1.45	34.5	3.4	6.6	1.15	1.6	0.28
34b	88.4	5.4	6.2	8.9	0.03	1.43	33.5	3.0	7.3	1.26	1.6	0.28
35a	84.6	10.0	5.4	8.4	0.24	1.45	34.0	3.6	9.1	1.58	2.0	0.35
35b	87.8	6.4	5.8	6.8	0.02	1.46	33.5	2.8	7.3	1.28	—	—
36a	83.8	6.4	9.8	9.2	0.47	1.46	34.5	4.0	8.3	1.46	1.8	0.32
36b	88.0	5.6	6.4	9.1	0.03	1.46	33.5	3.4	6.0	1.06	1.6	0.28
37a	62.8	14.4	22.8	28.6	2.10	1.42	52.0	16.8	24.5	4.19	7.1	1.21
38a	38.4	39.2	22.4	35.6	2.12	1.37	54.0	16.6	24.3	4.00	8.0	1.32
39a	59.6	25.0	15.4	16.4	1.52	1.54	41.0	11.2	16.5	3.06	4.5	0.83
39b	58.6	25.8	15.6	20.7	0.73	1.50	42.0	9.2	18.8	3.38	4.4	0.79
40a	61.6	23.4	15.0	21.8	1.83	1.41	44.0	11.6	18.3	3.10	5.0	0.85
41a	38.4	36.2	25.4	35.4	0.75	1.41	51.0	19.0	23.9	4.05	6.8	1.15
41b	29.8	34.4	35.8	53.8	0.71	1.41	54.0	21.6	26.0	4.41	8.0	1.36
42a	44.0	36.5	19.5	31.0	1.70	1.33	50.5	17.0	22.0	3.52	—	—
42b	40.0	42.6	17.4	29.8	0.74	1.38	48.5	15.4	24.0	3.97	5.4	0.89

* Each of these two moisture contents is expressed in two ways: first, in percentage dry weight of soil; and second, in inches of water per foot of soil.

** At each location, samples were taken at depths of (a) 4 to 8 inches and (b) 10 to 14 inches. Samples containing gravel were discarded.

APPENDIX—Continued
TABLE 4.—SUMMARY OF SOILS DATA—Continued

Sample No.	Sand %	Silt %	Clay %	Colloid %	Organic matter %	Volume weight	Settling volume cc.	Moisture equivalent %	Field capacity*		Wilting coefficient*	
									%	in.	%	in.
43a**	65.6	24.4	10.0	11.6	0.75	1.43	40.0	8.8	14.6	2.52	3.4	0.58
43b	59.4	31.0	9.6	18.0	0.67	1.51	41.0	8.2	16.5	2.99	3.5	-0.63
44a	24.6	34.0	41.4	58.4	0.85	1.21	54.0	21.8	31.0	4.49	9.5	1.38
44b	6.2	33.8	60.0	79.8	0.32	1.23	61.5	30.8	32.9	4.84	9.3	1.37
45a	27.2	41.4	31.4	42.6	0.62	1.37	51.5	17.8	26.5	4.35	7.3	1.20
45b	20.4	38.4	41.2	52.9	0.44	1.41	53.5	22.2	21.1	3.55	8.5	1.44
46a	26.0	27.4	46.6	58.2	1.32	1.28	60.0	24.0	28.6	4.42	—	—
46b	6.4	34.4	59.2	78.4	0.25	1.17	61.0	34.4	35.9	5.06	11.7	1.64
47a	31.6	35.2	33.2	52.2	0.83	1.20	56.0	23.4	30.1	4.36	9.1	1.31
47b	23.2	34.2	42.6	61.8	0.40	1.23	6.0	25.6	30.7	4.53	7.9	1.17
48a	36.8	38.0	25.2	36.6	1.60	1.27	54.0	18.0	27.3	4.16	8.5	1.30
48b	37.6	36.6	25.8	33.0	0.97	1.38	49.0	17.0	25.8	4.27	7.2	1.19
49a	60.6	32.2	7.2	17.0	1.10	1.46	43.0	9.2	18.2	3.18	3.6	0.63
49b	56.0	34.0	10.0	15.2	1.25	1.40	43.5	11.6	16.9	2.84	3.6	0.60
50a	58.8	32.2	9.0	16.5	1.03	1.44	43.0	9.4	16.1	2.78	4.7	0.81
50b	60.0	33.0	7.0	15.0	0.59	1.45	41.0	11.8	20.6	3.59	4.6	0.80
51a	65.6	25.1	9.3	15.2	0.95	1.48	38.0	7.4	12.3	2.18	2.6	0.46
51b	67.0	27.0	6.0	10.6	0.62	1.49	37.5	7.0	14.2	2.53	3.8	0.68
52a	65.6	25.0	9.4	15.4	—	1.41	39.5	9.0	15.8	2.69	3.3	0.56
53a	13.4	29.8	56.8	76.6	0.34	1.24	62.0	30.4	33.8	5.04	12.4	1.84
53b	10.0	18.8	71.2	83.8	0.02	1.22	64.0	35.0	34.7	5.11	13.3	1.95
54a	39.8	41.2	19.0	31.4	1.07	1.42	49.0	15.4	22.2	3.78	5.4	0.92
54b	12.6	53.6	33.8	60.0	0.77	1.30	57.0	25.6	30.9	4.84	—	—

* Each of these two moisture contents is expressed in two ways: first, in percentage dry weight of soil; and second, in inches of water per foot of soil.
** At each location, samples were taken at depths of (a) 4 to 8 inches and (b) 10 to 14 inches. Samples containing gravel were discarded.

FEEDING GRAINS OF DIFFERENT PROTEIN CONTENT TO GROWING PIGS¹

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In experiments reported recently from this Department (3) it was observed that when grain constituted the only source of protein in rations for rats and pigs, the protein content of the grains used had a marked influence on the rates of gain of both species. The results obtained from groups of pigs fed a mixed protein supplement at an arbitrary level without regard to the protein content of the grain in the basal ration, showed that the grain replacement value of a pound of protein supplement was almost 2.8 times greater when used with grains averaging 9.2 per cent in protein content than when added to grains containing 16.6 per cent protein. Data obtained from a pig feeding trial designed to study the effect of adjusting the level of protein supplementation with reference to the protein content of the grains in the basal ration are reported in the present paper.

EXPERIMENTAL

Selection and Analysis of Grains

Nitrogen determinations were done on a large number of samples of grain grown in different soil zones of Alberta in 1947. On the basis of these analyses, samples of oats and barley representing a wide range in protein content were selected and bulk quantities were purchased for the feeding trial. With a view to eliminating differences in feeding value which might be attributable to variety an attempt was made to select high, medium and low protein samples of one variety of each grain, but in the case of barley this was not possible, and, as is shown in Table 1, the high protein barley sample finally selected was of the Trebi variety, while the low and medium protein samples were Olli. The bulk quantities were resampled for analysis after delivery. Data for the protein content ($N \times 6.25$) of these grains obtained from two or more analyses in duplicate on 1 gram samples by the Kjeldahl-Gunning-Arnold method (1) using mercuric oxide as catalyst, and for crude fibre content as determined by the A.O.A.C. method (1), are summarized in Table 1.

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TABLE 1.—VARIETY AND ANALYSES OF GRAINS
(Basis 13.5 per cent moisture)

Grain	Variety	Protein	Fibre
		%	%
Low protein oats	Eagle	9.5	10.8
Medium protein oats	Eagle	11.5	10.5
High protein oats	Eagle	15.9	11.6
Low protein barley	Olli	9.1	6.1
Medium protein barley	Olli	12.2	6.6
High protein barley	Trebi	15.2	6.0

TABLE 2.—RATIONS
(During period A all groups were supplemented with feeding oil at a rate of 8 ml. per pig per day. The feeding oil contained 1200 I.U. vitamin A and 200 I.U. vitamin D per gram)

Feed	Grain + minerals + vitamins						Grain + minerals + vitamins + protein					
	1		2		3		4		5		6	
	L.P.		M.P.		H.P.		L.P.		M.P.		H.P.	
	A*	B*	A	B	A	B	A	B	A	B	A	B
Low protein oats, lb.	39	9	—	—	—	—	33	9	—	—	—	—
Low protein barley, lb.	59	89	—	—	—	—	51	81	—	—	—	—
Medium protein oats, lb.	—	—	39	9	—	—	—	—	36	9	—	—
Medium protein barley, lb.	—	—	59	89	—	—	—	—	54	86	—	—
High protein oats, lb.	—	—	—	—	39	9	—	—	—	—	37.75	9
High protein barley, lb.	—	—	—	—	59	89	—	—	—	—	56	87.50
Iodized salt, lb.	1	1	1	1	1	1	—	—	—	—	0.25	0.25
Ground limestone, lb.	1	1	1	1	1	1	—	—	—	—	—	—
Mixed supplement, lb.	—	—	—	—	—	—	16	10	10	5	6	3
Protein in ration, per cent**	9.1	9.0	11.7	11.9	15.2	15.0	13.8	12.0	14.5	13.4	16.8	15.9
Fibre in ration, per cent**	7.8	6.4	8.0	6.8	8.1	6.4	7.6	6.5	7.9	6.9	8.1	6.5

* Period A—start of experiment to 110 lb.; Period B—110 lb. to market weight, all groups.
** Basis 13.5 per cent moisture.

Feeding Trial

Purebred Yorkshire weanling pigs from the University of Alberta swine herd were used in this trial. Six lots of seven pigs each were made up to be comparable with respect to litter, sex and initial weight. They were housed in pens 10 ft. \times 12 ft. without access to soil or sunlight.

The feeding trial was divided into two periods: period A—30 to 110 lb.; period B—110 lb. to market weight. During period B the proportion of barley to oats in the ration was increased and the amount of protein supplement was decreased. The pigs were hand-fed three times daily until they reached an average weight of 110 lb. and twice daily thereafter.

The rations for lots 1, 2 and 3 were composed of low, medium and high protein grains, respectively, supplemented with iodized salt, ground limestone and feeding oil. For these lots the grains comprised the only source of protein in the ration. In addition to the grains, lots 4, 5 and 6 were given feeding oil and graded amounts of mixed protein-mineral supplement, depending upon whether the grains used were low, medium or high in protein. The following rates of supplementation were employed during periods A and B, respectively: lot 4, low protein grains, 16 per cent and 10 per cent; lot 5, medium protein grains, 10 per cent and 5 per cent; lot 6, high protein grains, 6 per cent and 3 per cent. In view of the small proportion of mixed supplement in the ration of lot 6, this group was given additional iodized salt and ground limestone. The feeding oil was omitted from the rations for all groups after the pigs reached an average weight of 110 pounds.

The mixed supplement used in the rations for lots 4, 5 and 6 contained 37.4 per cent protein and 5.6 per cent fibre on a 13.5 per cent moisture basis, and was composed of the following: tankage 50, linseed oil meal 25, fish meal 7, alfalfa meal 8, ground limestone 5 and iodized salt 5 pounds per hundred.

The rations fed are listed in detail in Table 2.

RESULTS AND DISCUSSION

A summary of the results of the feeding trial is presented in Table 3.

Reference to the results shown in Table 3 for lots 1, 2 and 3 shows that the protein content of the grains used had a marked effect on the rate of gain and feed consumption per unit gain when the grains comprised the only source of protein in the ration. These data are in good agreement with those of McElroy, Lobay and Sinclair (3). The rate of gain for lot 1, fed low protein grains, was 0.35 lb. per day slower than that for lot 3, fed high protein grains, and the feed consumption per 100 lb. gain was 24 per cent greater. Lot 2, fed medium protein grains, was intermediate between lots 1 and 3 in rate and economy of gain.

That a substantial reduction in the amount of supplementary protein required in swine rations may be possible, dependent upon the protein content of the cereal portion of the ration, is shown by the results obtained for lots 4, 5 and 6. Though there were no marked differences between the results for these lots it is significant that the pigs of lot 6, fed a ration in which only 10 per cent of the total protein was contributed by the mixed supplement, made slightly faster gains and more efficient use of feed than

TABLE 3.—LOW, MEDIUM AND HIGH PROTEIN GRAINS FOR PIGS

Lot number	1	2	3	4	5	6
Description of ration	Grain + minerals + vitamins			Grain + minerals + vitamins + protein		
	L.P.	M.P.	H.P.	L.P.	M.P.	H.P.
Mean protein in ration, per cent	9.1	11.8	15.1	13.0	14.0	16.4
Protein from:						
Grain, per cent	100	100	100	62	80	90
Mixed supplement, per cent	—	—	—	38	20	10
Number of pigs in lot	7	7	7	7	7	7
Average initial weight, lb.	28.3	28.4	29.0	29.3	29.1	29.8
Average final weight, lb.	196.3	200.6	203.9	207.8	204.0	204.8
Average number days on experiment	208	183	151	137	141	132
Total gain, lb.	1176	1205	1224	1250	1224	1225
Average daily gain, lb.	0.81	0.94	1.16	1.30	1.24	1.32
Average daily feed, lb.	3.72	3.82	4.31	4.81	4.78	4.64
Feed required for 100 lb. gain:						
Grain, lb.	451.8	398.1	364.7	324.1	358.5	334.2
Mixed supplement, lb.	—	—	—	45.2	27.0	14.5
Ground limestone, lb.	4.6	4.1	3.7	—	—	0.5
Iodized salt, lb.	4.6	4.1	3.7	—	—	0.8
Total, lb.	461.0	406.3	372.1	369.3	385.5	350.0
Average Advanced Registry Carcass Score, per cent	37.3	57.6	65.0	71.0	65.7	70.7

did the pigs of lots 4 and 5, fed rations in which 38 per cent and 20 per cent, respectively of the total protein was derived from the supplement. The results for lots 4 and 6 show that 30.7 lb. of mixed supplement were saved at a cost of 10.1 lb. of grain, a saving attributable to the high protein content of the grains fed to group 6.

The data for lot 3, fed a ration containing 15.1 per cent protein of grain origin, confirm the knowledge that the quality of cereal proteins is inadequate to support optimum growth in swine. It appears, however, that if the grains contain a sufficient quantity of protein to provide a substantial amount of plant protein in the ration, good results can be obtained by supplementation with relatively small amounts of high quality protein.

Annual reports of the Board of Grain Commissioners for Canada (2), in which the protein content of carlot samples of barley selected from different crop districts in Western Canada is reported, indicate that in certain areas a considerable volume of the barley produced contains either less than 10 per cent or more than 14 per cent protein. The results of the experiment described in this paper suggest that when grains of high protein content are available the usual recommendations regarding rates of protein supplementation may be revised downward. Conversely, although additional experiments are required to show whether it is likely to be economical to fortify grains of low protein content with unusually high levels of supple-

ment, the results of this and a previously reported study (3) emphasize the special value of protein supplement in swine rations based on low protein grains.

Representatives of the Production Service of the Dominion Department of Agriculture co-operated in cutting and scoring the carcasses from this experiment by the Advanced Registry method. It is shown in the last line of Table 3 that the average carcass score for group 1 fed low protein grains, supplemented simply with ground limestone, salt and feeding oil, was only 37.3 per cent. These pigs were on test for 7 months and were about 9 months old when marketed at 200 pounds. The detailed score showed that their carcasses were, if anything, a little shorter than those of the other groups; they were also fatter as indicated by depth of fat at shoulder, back and loin and by a high dressing percentage. The muscles of these pigs, fed a ration deficient in both amount and quality of protein, were small and poorly developed, as shown by the area of the eye of lean and by the low belly score which averaged only 44.3 per cent of the maximum obtainable under the Advanced Registry system of scoring. These results for lot 1, as well as those for lot 2, simply emphasize the well-known fact that pigs that are fed unbalanced rations and grow slowly all the way from weaning to market weight are likely to yield poor carcasses.

SUMMARY

An experiment is described in which the feeding value for swine of grains of high, medium and low protein content was studied. The results confirm those reported in a previous paper in which it was shown that when grains constitute the only source of protein in the ration, the rate and economy of gain in pigs is markedly influenced by the protein content of the grains employed. It is also shown that when high protein grains are used in the ration, good results can be obtained by adding substantially smaller quantities of protein supplement than are required when the grains used are of low protein content.

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ERADICATION OF POISON IVY (*RHUS RADICANS* L.)

IV. EXPERIMENTS WITH AMMONIUM SULFAMATE AND SODIUM CHLORATE¹

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Investigations were initiated at Ottawa, Ontario, in 1941 to test the herbicidal properties of ammonium sulfamate on poison ivy. Their purpose was three-fold: (A) to compare the effectiveness of ammonium sulfamate and sodium chlorate, (B) to investigate the relationship between dosage and control, and (C) to determine the best time of year for application. The results reported here are from plots treated in 1942 and 1943, together with follow-up treatments made on some of these plots in 1944 and 1945.

REVIEW OF LITERATURE

Preliminary tests to evaluate sulfamates as weed killers were reported by Cupery and Cupery (6) in 1939. Poison ivy was one of the plants under test and these investigators observed that it was particularly sensitive to ammonium sulfamate applied as a spray. Dietz, Vogel, and Cupery (7) recorded further observations on these 1939 test plots and gave results obtained from treatments made in 1940. They could find no noticeable difference in ultimate efficiency between ammonium sulfamate and sulfamic acid. The rapidity of action of ammonium sulfamate was found to be influenced by humidity and rainfall, and to some extent by temperature. They concluded that ammonium sulfamate properly applied was an effective weed killer for poison ivy. Yeager and Calahan (16) gave the results of two years' tests on poison ivy in which sodium chlorate was compared with ammonium sulfamate. Sodium chlorate applications gave a very poor control whereas those with ammonium sulfamate gave an almost complete kill. Southwick (12) studied the effect of ammonium sulfamate applications on poison ivy growing under apple trees. Concentrations of $\frac{1}{2}$, $\frac{3}{4}$, and 1 pound per gallon of water killed the ivy foliage but some recovery occurred the following year. Flory (8), Grigsby (9), and Jacques and Meilleur (10) secured a complete kill of poison ivy with applications of ammonium sulfamate. Their reports refer, however, only to the year in which the materials were applied and do not include observations made in the year following the treatment. Cross (5) mentioned very briefly the effect of ammonium sulfamate sprays on poison ivy in cranberry bogs.

Although it was first investigated as a herbicide in 1939, ammonium sulfamate was reported to have received widespread tests as an eradicator for poison ivy in 1941, 1942, and 1943 (1, 2). Reports of community-sponsored campaigns to eradicate poison ivy with ammonium sulfamate have been published by Tapley (13) and Towle (14).

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MATERIALS AND METHODS

In the present experiments, the plots, each 100 square feet in area, were on open ground in the vicinity of Ottawa West, Ontario. The soil was a very shallow Farmington loam over limestone bedrock. Stones were numerous on the surface of many of the plots. Poison ivy was quite thick, with an average ground cover of 80 per cent and upright stems ranging from 18 to 24 inches in height. Although the areas suitable for the experiments were rather small, they were in close proximity and were similar in respect to poison ivy cover. All applications were made to the ivy foliage with a Brown's No. 4 "Open-hed" sprayer. Treatments were made in two successive years rather than being replicated in any one year.

Estimates of the percentage cover of poison ivy were made visually before and after each application. They represent ground cover, that is to say, the percentage of the area of the plot covered with poison ivy, but they do not take into account the density of the foliage in the completely covered areas.

The ammonium sulfamate was relatively pure and was secured from the Grasselli Chemicals Department of E. I. du Pont de Nemours & Co., through the courtesy of B. L. Emslie of Canadian Industries Ltd., Montreal. The sodium chlorate was purchased from the Electric Reduction Sales Co., Ltd., of Toronto.

EXPERIMENTS AND RESULTS

Rate of Application

Other conditions being equal, dosage—the quantity of toxicant applied per unit of area—may be changed by varying gallonage or concentration. In this experiment, gallonage—the amount of herbicidal solution—was kept constant and the concentration of the solution was varied. The gallonage used was one Imperial gallon per 100 square feet. This gallonage permitted thorough spraying of the plots in two directions. A certain amount of run-off occurred. The concentrations investigated were 10, 6.7, 5, and 4 per cent. The 1942 treatments were applied between 1.30 and 3.00 p.m. on June 9, and those of 1943, between 2.00 and 4.00 p.m. on June 18. Since up to the time that the 1943 applications were made, no poison ivy had yet recovered on any of the plots sprayed with ammonium sulfamate in the previous year, a fifth and weaker concentration (3.3 per cent) was also applied. In addition, to obtain preliminary information on the effect of decreasing the gallonage of ammonium sulfamate, applications of $\frac{1}{2}$ gallon of 5 and of 10 per cent solution were included in the 1943 experiment. These treatments were made on an adjacent area of poison ivy on June 16, 1943, between 3.00 and 3.45 p.m. The amounts of materials used in the various treatments are given in Table 1.

The season in 1942 was earlier than in 1943, so that the plants were in approximately the same stage of growth, and therefore phenologically comparable on the respective dates of application. Clear and sunny weather prevailed on the days the herbicides were applied. In 1942 the weather was dry whereas in 1943 it was much wetter than average (Table 6). Rainfall, with the amount in inches given in brackets, was recorded for the

TABLE 1.—QUANTITY OF MATERIAL AND EQUIVALENT COVERAGES FOR THE DIFFERENT HERBICIDAL APPLICATIONS

Dosage		Weight of herbicide	Equivalent coverage for 1 pound of herbicide	Equivalent number of pounds per acre
gal.	conc. %	oz.	sq. ft.	lb.
1	10	16	100	436
1	6.7	10.6	150	290
1	5	8	200	218
1	4	6.4	250	174
1	3.3	5.3	300	145
0.5	10	8	200	218
0.5	5	4	400	109

period immediately before and after the applications, as follows: 1942—June 6 (0.15) and June 13 (0.42); 1943—June 15 (1.73), 17 (0.58), 19 (0.05), 26 (0.33), and June 27 (1.30).

Table 2 gives the results obtained from the applications of the five different concentrations. Figure 1 gives the dosage-response curves obtained when percentage reduction of poison ivy is plotted against the logarithm of these concentrations.

With ammonium sulfamate, the control of poison ivy varied from good to excellent for the 1942 applications and from fair to good for those made in 1943. With sodium chlorate, the control varied from fair to good

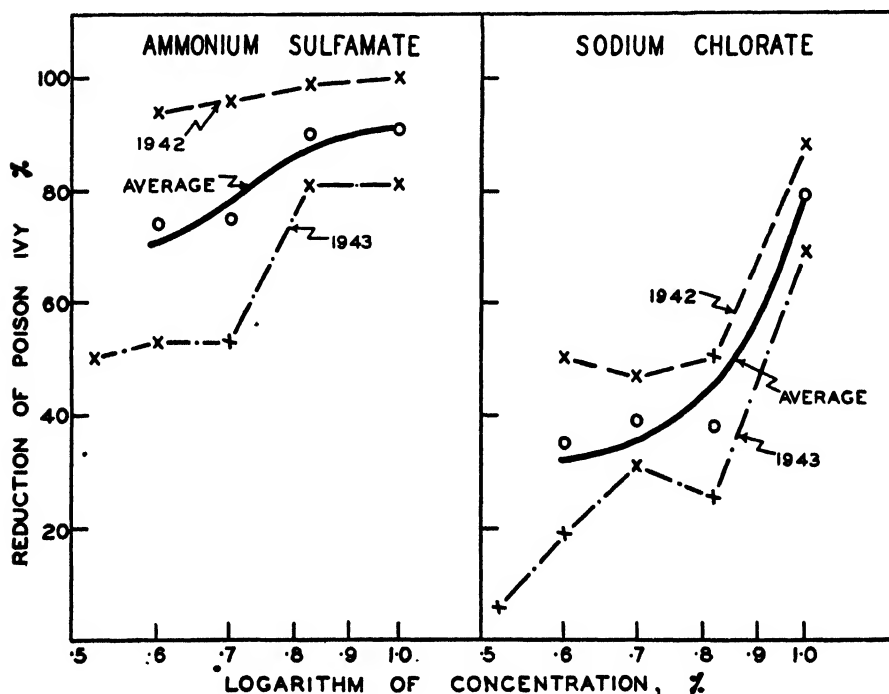


FIGURE 1. Dosage-mortality curves, showing the effect of ammonium sulfamate and sodium chlorate on poison ivy (the dosages were 1 gallon per 100 sq. ft. of 3.3, 4, 5, 6.7, and 10 per cent solutions).

for the 1942 applications and from poor to fair for those made in 1943. Although in 1942 the level of control obtained with all concentrations of both herbicides was higher than for the corresponding concentration in 1943, the slope of the curves for both years was somewhat similar for increase of concentration in the sprays. While, therefore, some factor caused a different level of control in the two years, the effect of increasing the concentration, i.e., the relationship of dosage to control, was approximately the same in both years.

A significant difference in the control of poison ivy from increase of concentration was evident between ammonium sulfamate and sodium chlorate. With ammonium sulfamate, the control from concentrations of 4 and 5 per cent was only slightly inferior to that obtained from concentrations of 6.7 and 10 per cent. With sodium chlorate, however, the control from concentrations of 4, 5, or 6.7 per cent was definitely inferior to that obtained from the 10 per cent solution. While the slope of the curve, therefore, rises rapidly from the 6.7 to the 10 per cent concentration for sodium chlorate it levels off at these concentrations for ammonium sulfamate (Fig. 1).

TABLE 2.—EFFECTS OF AMMONIUM SULFAMATE AND SODIUM CHLORATE APPLIED AS FOLIAGE SPRAYS TO POISON IVY ON JUNE 9, 1942, AND ON JUNE 16 AND 18, 1943

Dosage		Percentage reduction in July of the second year after application					
		Ammonium sulfamate			Sodium chlorate		
		1942 application	1943 application	Average	1942 application	1943 application	Average
gal.	conc. %	%	%	%	%	%	%
1	10	100	81	91	88	69	79
1	6.7	99	81	90	0	25	38
1	5	96	53	75	47	31	39
1	4	94	53	74	50	19	35
1	3.3	—	50	—	—	6	—

Time of Application

To determine the best time of year to apply herbicides for the control of poison ivy, a single application of ammonium sulfamate and of sodium chlorate was applied as a spray in each of the four months—June, July, August, and September—of 1942 and 1943. The dosage selected was 1 gallon of a 10 per cent solution for each plot. In 1942, a grass fire overran the experimental area during the first week of August making it impossible to carry out the August and September treatments. Previously, however, treatments had been made on June 8 and July 15. The 1943 treatments were made on June 16, July 13, August 6, and September 8. All treatments were made in the early afternoon (2.00-3.00 p.m.) except those of July, 1942, which were made at 7.00 p.m.

All days on which treatments were applied were clear and sunny. Rainfall was recorded on the day following the June, August, and September treatments in 1943, but following the treatments in June and July, 1942, and in July, 1943, a 2- to 6-day interval elapsed before any rain fell. In

1943, owing to the high rainfall, the poison ivy in the plots remained in an active state of growth much later than usual. As a result, the foliage was dark green in colour throughout most of August and only one-quarter of the leaves were assuming their autumnal coloration by September 8. This condition was in marked contrast to that in 1942, when, owing to dry weather, some of the ivy leaves were turning a red or yellow colour at the time of the July treatment.

To obtain information on the efficiency of these herbicides when used in follow-up treatments, it was proposed to continue the applications each year, and in the same month as the original treatment, until the poison ivy was eradicated. Unfortunately a new housing sub-division was opened up in 1946 in the vicinity of the plots and it was necessary to abandon the experiment before final treatments could be made.

Table 3 gives the differences in control obtained from the applications of ammonium sulfamate and sodium chlorate made in June, July, August, and September. With ammonium sulfamate, there was no significant difference between the control obtained from applications made in June or July. Applications made in both these months gave excellent control and resulted in a greater reduction of poison ivy than was obtained from applications made in August or September. With sodium chlorate, the control was good, fair to poor, poor, and zero for applications made in June, July, August, and September, respectively. Not only did applications of ammonium sulfamate give better control of poison ivy than did those of sodium chlorate but the results indicated that the period of time during the growing season in which good control can be expected is longer for ammonium sulfamate than for sodium chlorate treatments.

TABLE 3.—EFFECTIVENESS OF AMMONIUM SULFAMATE AND SODIUM CHLORATE ON POISON IVY WHEN APPLIED AS FOLIAGE SPRAYS IN EACH OF JUNE, JULY, AUGUST, AND SEPTEMBER

Herbicide	Month	Percentage reduction in July of first year after application	
		1942 application	1943 application
Ammonium sulfamate (1 lb. in 1 gal. water per 100 sq. ft.)	June	% 99	% 94
	July	93	98
	Aug.	—*	53
	Sept.	—*	78
Sodium chlorate (1 lb. in 1 gal. water per 100 sq. ft.)	June	95	78
	July	29	67
	Aug.	—*	29
	Sept.	—*	0

* Grass fire removed foliage from the plots before treatment could be made.

In the follow-up treatments consisting of one herbicidal application per year for 3 successive years, the June and July applications again gave the best results with both ammonium sulfamate and sodium chlorate (Table 4). Complete eradication was obtained from 2 and 3 years of treatments, respectively, for applications of ammonium sulfamate made in June and July. To reduce a dense stand of poison ivy to a few scattered

TABLE 4.—THE EFFECT OF REPEATING HERBICIDAL APPLICATIONS ONCE EACH YEAR FOR THREE YEARS (AVERAGE RESULTS FOR TWO TREATMENTS)

Herbicide	Month of treatment	Average ground cover of poison ivy			
		Year of treatment			Year following completion of treatment
		1	2	3	1
		%	%	%	%
Ammonium sulfamate (1 lb. in 1 gal. water per 100 sq. ft.)	June	85	3*	0	0
	July	85	4	1	0
	Aug.	85	50	1	1
	Sept.	85	35	10	2
Sodium chlorate (1 lb. in 1 gal. water per 100 sq. ft.)	June	85	12	2	1
	July	80	45	5	1
	Aug.	85	60	55	8
	Sept.	70	70	65	50

* Due to the small size of the plants in the second year it was necessary to defer treatment until July.

plants required 1, 1, 2, and 3 years, respectively, for applications of this chemical made in June, July, August, and September. The total amounts of herbicide required to bring about this reduction were 1.0, 1.0, 2.0, and 2.5 pounds, respectively. With sodium chlorate, complete eradication was not obtained from any of the treatments for the three successive years. The applications made in June and July reduced a dense stand of ivy to a few scattered plants, the 2 and 3 years of treatment requiring 1.7 and 2.5 pounds of chlorate, respectively. The August treatments reduced the poison ivy a considerable amount but, even after a total of 3 pounds of chlorate had been applied in the 3 applications, a fair number of clumps were still present. The chlorate sprays in September did not reduce the stand of poison ivy an amount sufficient to be considered satisfactory control. Although it was not possible to carry this part of the experiment through to its conclusion, it is quite evident that fewer treatments were required with ammonium sulfamate than with sodium chlorate to eradicate completely poison ivy and best results with both herbicides were obtained from applications made in June or early July.

A Comparison of the Effect of Ammonium Sulfamate with that of Sodium Chlorate

From the results obtained under rate of application and time of application, it is quite evident that, under comparable conditions and on a basis of equal weight, better control was obtained with ammonium sulfamate than with sodium chlorate. It was also noted during the experiments that ammonium sulfamate killed a greater proportion of the grass than did sodium chlorate.

The action of ammonium sulfamate and sodium chlorate on the poison ivy foliage was quite similar. Following the application of the solution to the foliage, the leaves gradually turned brown and became curled, dry, and crisp. After treatments with concentrations of 5 or 10 per cent, the leaves died within 5 to 7 days.

TABLE 5.—THE RECOVERY OF POISON IVY FOLLOWING TREATMENT WITH AMMONIUM SULFAMATE AND SODIUM CHLORATE

Herbicide	Dosage	Date of treatment	Ground cover of new growth poison ivy				
			During year of application			First year after application	Second year after application
			July 15	Aug. 16	Oct. 1	July	July
	gal. conc. %		%	%	%	%	%
Ammonium sulfamate	1 10	June 9/42	0	0	0	0	0
	1 10	June 18/43	0	0	0	5	15
	1 5	June 9/42	0	0	0	2	3
	1 5	June 18/43	0	0	0	9	35
Sodium chlorate	1 10	June 9/42	1	4	4	8	10
	1 10	June 18/43	1	4	5	20	25
	1 5	June 9/42	2	15	30	35	40
	1 5	June 18/43	3	5	15	45	55

The rate of recovery of the poison ivy following the treatments differed markedly for the two herbicides, as will be seen from Table 5. On the plots sprayed with sodium chlorate, new green leaves were appearing within one month after the treatment, whereas, on those sprayed with ammonium sulfamate, new green leaves did not appear until the summer following the treatment. In none of the treatments made to date with ammonium sulfamate has the author yet observed new growth of poison ivy during the year of treatment, except when weak concentrations, such as 3.3 or 4.0 per cent, were applied—and then only in certain years. The new leaves that developed in the sodium-chlorate treated plots arose from buds on the existing upright stems. They grew quite rapidly. In the ammonium-sulfamate treated plots, however, the new leaves that appeared in the summer following the application were on new stems, sucker growth, and arose direct from the underground root-stalks. These stems were, of course, quite small and some of them did not appear until late in July.

The Possible Effect of Rainfall on the Control of Poison Ivy

In these experiments, treatments made in June, 1942, both with ammonium sulfamate and with sodium chlorate gave better control of poison ivy than did those made in June, 1943. From rainfall data collected at Ottawa by the Field Husbandry Division of the Central Experimental Farm (Table 6), it will be seen that, in 1942, June and July were drier than average, while May and August had approximately an average amount of rainfall. In 1943 although July was quite dry, May, June, and August were much wetter than average. Therefore, the rainfall, and consequently the soil moisture, differed markedly in these two years and might have accounted for the difference in control. Also in 1943, when the July application of ammonium sulfamate gave a slightly better control of poison ivy than that made in June (Table 3)—the opposite of the results usually obtained—July was much drier than was June. Heavy

TABLE 6.—RAINFALL AT OTTAWA, ONTARIO

Month	Fifty-three year average	1942	1943
	inches	inches	inches
April	2.3	1.5	2.4
May	2.7	2.8	4.6
June	3.4	2.0	5.7
July	3.7	2.3	2.2
August	3.0	2.9	9.1
September	2.9	6.5	1.7
October	2.7	2.4	4.3

showers of rain fell during the day previous to and the day following the June application but in July no rain fell for 8 days before or 7 days after the treatment.

Considerable difference was noted in these two years in respect to the effect of ammonium sulfamate on the thin cover of grass present on the plots. All of the 1942 treatments, and to a lesser extent the July treatment in 1943, destroyed most of the grass that was present. In comparison, the June and August treatments in 1943, made during periods of heavy rainfall, had no harmful effect.

Robbins *et al.* (11) state that sodium chlorate can affect plants in different ways: it may act as a contact poison on plant leaves; following its absorption by the leaves, it may serve as a translocated herbicide under certain conditions; and it may kill plants by root absorption. Under conditions of the present experiments, with 1 gallon of solution per 100 square feet, there was run-off on to the soil so that there was opportunity for action both as a translocated spray and as a soil sterilant.

These authors (11) investigated the effect of soil moisture on the translocation of sodium chlorate and obtained a better kill of wild morning-glory (*Convolvulus arvensis*) when the soil was dry than when wet. Apparently a high water deficit in the plant brought about better translocation and more complete distribution of toxicant within the roots. Crafts (4) studied the distribution of chlorate in the soil as affected by leaching. His results indicate that chlorate is readily leached downward by water in the soil and that its distribution is largely determined by the amount of water that passes into and through the soil after the chemical is applied. As the soil in the area of the present experiments was prevailingly shallow, the high rainfall in 1943 may have leached the herbicide away from the underground parts of the plants before it could exert its maximum killing power. Therefore, whether the killing of poison ivy by sodium chlorate is due to translocation of the material or to its acting as a soil sterilant, or to a combination of both, it is quite possible that the high rainfall in 1943 accounted for the lower percentage reduction of poison ivy.

That factors other than soil moisture may affect the control of poison ivy when ammonium sulfamate is applied as a herbicide is evident from Table 7. The results given in Table 7 were obtained when different gallonages and concentrations were applied to different locations on June

16 and 18, 1943. A better control of poison ivy was obtained on plots 11 and 12 than on plots 2 and 6 although the former plots received one-half the quantity of solution, and therefore one-half the actual weight of sulfamate. A greater reduction of poison ivy occurred in plot 12 than in plots 2, 6, 8, or 10 although each of these plot received a greater amount of herbicide than did plot 12.

TABLE 7.—THE CONTROL OF POISON IVY WHEN DIFFERENT DOSAGES OF AMMONIUM SULFAMATE WERE APPLIED TO DIFFERENT LOCATIONS AT DIFFERENT DATES

Plot	Date of application	Dose			Reduction of poison ivy
		Gallonage	Concentration	Weight	
	1943	gal.	%	oz.	%
11	June 16	0.5	10	8	98
2	June 18	1.0	10	16	81
12	June 16	0.5	5	4	88
6	June 18	1.0	5	8	53
10	June 16	1.0	3.3	5.3	50
8	June 18	1.0	4	6.4	53

It is not definitely known what factor or factors were responsible for these differences in control but at least two possible causes are worth mentioning. The size of the upright stems of poison ivy differed in the two areas and there was a difference in the amount of rainfall immediately following the applications. Although the areas were in close proximity and the difference in the percentage ground cover of poison ivy was small, there was a denser stand, with higher and coarser stems, on the area containing plots 10, 11, and 12 than on the area containing plots 2, 6, and 8. The area containing plots 10, 11, and 12 was sprayed June 16. On the previous day, 1.73 inches of rain fell and, on the day after the treatment 0.58 inches. The area containing plots 2, 6, and 8, was sprayed on June 18. On the previous day, 0.58 inches of rain fell, but, after the treatment, except for 0.05 inches on the second night, no rain fell until the eighth day. The best control, therefore, was obtained in the plots with the coarsest stems and on which rain fell during the day following the treatments.

While there may be some relationship between the amount of rainfall and the control of poison ivy with ammonium sulfamate or sodium chlorate, experimental work under controlled conditions is required before the importance of rainfall or other environmental factors can be assessed.

DISCUSSION

Insufficient observations were made in these experiments to warrant the drawing of regression lines by plotting the results on logarithmic probability paper, as suggested by Wilcoxon and McCallan (15), or to plot transformed dosage-mortality curves according to Bliss (3). When plotted on semi-logarithmic paper, however, the dosage-response curves for ammonium sulfamate and sodium chlorate (Figure 1) show indications of the sigmoid character found in toxicity studies by many biologists.

The dosage-response curves make possible an evaluation of the recommendation for the eradication of poison ivy of 1 pound of ammonium sulfamate or sodium chlorate per 100 square feet. The levelling-off of the curve with ammonium sulfamate at concentrations of 6.7 and 10 per cent indicates that an increase of the concentration of this material, in the pure salt form, above 7 per cent would give little or no benefit as regards the control of poison ivy. With ammonium sulfamate, therefore, 1 pound per 100 square feet gives a considerable margin of safety and the amount of chemical could be lowered somewhat by either decreasing the concentration or the gallonage. Since, with sodium chlorate, the slope of the curve rises rapidly from the 6.7 to the 10 per cent concentration, definite advantages would be obtained from using a 10 per cent solution. Unfortunately, higher concentrations of sodium chlorate were not included in these experiments, so that it is not known whether the slope of the curve would continue to rise for concentrations just above 10 per cent or whether it would level off. With sodium chlorate, therefore, 1 pound per 100 square feet should be considered a minimum dosage.

From the results obtained, it is evident that, on a basis of equal weight, ammonium sulfamate gives a far better kill of the upright stems of poison ivy than does sodium chlorate. Because of this difference in degree of killing of the upright stems, it was difficult to make comparable estimates of poison ivy recovery. After the application of sodium chlorate, some of the upright stems of poison ivy recovered and put forth new leaves within 3 to 4 weeks. Accurate estimates of recovery, therefore, were possible in the first summer following the treatment. With ammonium sulfamate, however, because of the complete kill of upright stems, new growth did not appear until the next year, and then it came as sucker growth from the underground root stalks. As this new growth was quite small (some of it did not come through the ground until late in July), accurate estimates of recovery were not possible until the second summer after treatment. With ammonium sulfamate treatments, it was necessary to watch the area for at least two years after the application was made in order to make certain that regrowth of poison ivy did not occur.

Differences in time of recovery following the application also affected the timing of follow-up treatments. With sodium chlorate, it was possible to make a second application in the fall of the first year of treatment. With ammonium sulfamate, however, it was necessary to delay the follow-up treatment until the second or third year. In the sulfamate plots where the initial control was only fair, it was possible to make a follow-up treatment in the summer of the first year after the initial application. In those areas, however, where the initial control was excellent, the follow-up treatment could be delayed with advantage until the second summer after the initial application.

SUMMARY

Ammonium sulfamate and sodium chlorate were investigated as eradicans for poison ivy.

With ammonium sulfamate, the average reduction of poison ivy from June applications was 74, 75, 90, and 91 per cent for solution concentrations of 4, 5, 6.7, and 10 per cent, respectively. Applications made in July gave

almost as good a control as those made in June. Treatments made with a 10 per cent sulfamate solution in August or September gave a fairly good control but were less effective than those made in June or July.

With sodium chlorate, the average reduction of poison ivy from June applications was 35, 39, 38, and 79 per cent for the same respective solution concentrations. For treatments made with a 10 per cent chlorate solution in June, July, August, and September, the control was good, fair to poor, poor, and zero, respectively.

Very rarely was a complete kill of poison ivy obtained from a single application of either herbicide. Follow-up treatments in two or more years were required to eradicate the ivy. Eradication was achieved with less material and fewer treatments when the applications were made in June or early July. In the follow-up treatments, fewer applications were required with ammonium sulfamate than with sodium chlorate to give complete eradication.

After treatment with both herbicides, the poison ivy foliage turned brown and died within from 5 to 7 days. Some of the plants sprayed with sodium chlorate put forth new leaves within one month following treatment, but, on those treated with ammonium sulfamate, new growth did not appear until the following summer. On the sodium chlorate plots, the new growth arose from existing upright stems, while, on the ammonium sulfamate plots it came direct from underground rootstalks.

The most pronounced reduction of poison ivy was obtained under conditions of low rainfall.

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THE RELATIONSHIP OF CLEAN FLEECE WEIGHT TO FIBRE THICKNESS¹

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INTRODUCTION

The production of a maximum amount of clean wool per sheep is of paramount importance as this is the basis on which gross returns are determined. Although wool growers usually receive a higher price per clean pound for fine wool than for the coarser grades, production of fine wool is not necessarily the most remunerative. Greater amounts of coarser wool per sheep at slightly lower prices may result in larger total returns.

While there are many factors involved in wool improvement the most vital to the producer are those directly associated with greater wool production per sheep. These can be summed up in the term clean fleece weight, which in turn is dependent upon grade (i.e. fibre thickness), length of staple, density of fibres on the skin, and body size. The present study was undertaken to determine the relationship of clean fleece weight to fibre thickness, to ascertain whether selection on this basis of fibre thickness would be effective in the improvement of clean fleece weights.

REVIEW OF LITERATURE

Relatively little information is available on the actual relationship of clean fleece weight to grade, i.e. fibre thickness. However, several investigators have reported data in an effort to ascertain its economic significance. Burns (1) found that the coarser fleeces studied at the University of Wyoming weighed heavier and produced more clean wool per sheep than the finer fleeces. The average clean fleece weight of mature range ewes was 3.7 pounds for fine staple while one-half blood and three-eighths blood staple were 4.4 and 5.5 pounds, respectively. In the case of rams the one-half blood fleeces averaged 5.2 pounds and the one-quarter blood 8.4 pounds of clean wool per head. Results obtained from an analysis of 3,482 fleeces by Pohle and Keller (4) indicated a similar situation. Clean fleece weights for mature ewes increased from 4.3 pounds for fine French combing through the various grades to 6.6 pounds for one-quarter blood staple. For yearling ewes the range was from 3.3 to 5.9 pounds for the same grades. Spencer, Hardy, and Brandon (5) concluded from a study of the wool production of range Rambouillet ewes that as the fibres became coarser there was a slight tendency for the clean fleece weights to increase. Their data showed that the clean wool per fleece increased from 3.0 to 4.4 pounds, the grades being 68's and 56's, respectively.

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MATERIALS AND METHODS

Clean Fleece Weights

In 1946, 809 individual grease fleece samples were collected from experimental flocks in Western Canada and the Central Experimental Farm, Ottawa. These wool samples were scoured and the yields ascertained (basis 16 per cent regain) in accordance with standard laboratory procedure (6) to determine the clean content of each fleece.

Fibre Thickness

Each scoured sample was zoned and a small random sub-sample drawn for fibre thickness measurement. A cross-section was prepared from this material by means of the Hardy Thin Cross-Section Device (2) and with the aid of a microprojector projected on to a frosted glass screen at a magnification of $500\times$ (3). Two hundred fibres per sample were then measured, using a bi-diameter scale.

RESULTS AND DISCUSSION

The average clean fleece weights by grades for the fleeces analysed in this study are summarized in Table 1.

TABLE 1.—AVERAGE CLEAN FLEECE WEIGHTS BY GRADES

Grade	Number of fleeces	Average clean fleece weights
Fine staple	461	3.9
One-half blood staple	192	4.5
Three-eighths blood staple	68	4.9
One-quarter blood staple	62	4.8
Low one-quarter blood staple	19	5.6
Common and braid	7	5.7

It is apparent from these data that there is a general relationship between average clean fleece weights and fibre thickness expressed as commercial grades. The average clean weights increased from 3.9 to 5.7 pounds from the finest to the coarsest grades or a difference of 1.8 pounds. These results are in general agreement with those obtained by Burns (1) and Pohle and Keller (4), and indicate that as fibre thickness increases clean fleece weight has a tendency to increase. However, this is a general relationship and presumably arises out of the fact that fibres tend to increase in length as they become coarser and in addition breed effects enter into the picture over the whole range of grades considered.

More important from the standpoint of the breeder of one breed of sheep is the question of whether the same relationship holds true within the breed where the range of fineness normally is not so great. To partially answer this question a further, more detailed analysis was made of the data. To eliminate the effect of location, management, feed, breed or cross, and sex differences the data were analysed for each group separately as shown in Table 2.

TABLE 2.—AVERAGE CLEAN FLEECE WEIGHTS, AVERAGE FIBRE THICKNESSES AND COEFFICIENTS OF CORRELATION BETWEEN CLEAN FLEECE WEIGHT AND FIBRE THICKNESS BY BREEDS AND CROSSES, SEX, AND AGE

Source, breed or cross, sex, and age ¹	Number of fleeces	Average fibre thickness (microns)	Average clean fleece weight (lb.)	Coefficient of corre- lation	Probable error
<i>Dominion Experimental Station, Lethbridge</i>					
Rambouillet rams	27	20.6	4.2	0.093	±0.129
Rambouillet ewes	88	20.2	3.7	0.054	±0.072
Can. Corr. rams	14	22.6	4.0	0.314	±0.162
Can. Corr. ewes	30	20.9	3.4	0.397*	±0.104
N.Z. Corr. rams	10	24.5	4.7	0.898**	±0.041
N.Z. Corr. ewes	8	23.4	4.0	0.170	±0.169
F1 ewes (N.Z.C. × Ramb.)	16	21.0	3.9	0.135	±0.166
C2 ewes (C.C. × F1)	5	20.2	4.0	-0.211	±0.107
C3 rams (C.C. × C2)	12	22.8	4.6	0.420	±0.160
C3 ewes (C.C. × C2)	41	21.2	3.9	0.163	±0.120
C4 rams (C.C. × C3)	9	23.6	4.2	0.716*	±0.110
C4 ewes (C.C. × C3)	17	22.6	4.0	0.301	±0.148
<i>Dominion Range Experiment Station, Manyberries</i>					
Romnelet rams	69	23.9	4.2	-0.071	±0.081
Romnelet rams (m)	12	27.5	5.5	-0.236	±0.184
Romnelet ewes	72	25.8	4.6	0.156	±0.078
<i>Dominion Experimental Station, Swift Current</i>					
Rambouillet rams	22	21.3	4.8	0.177	±0.139
Rambouillet ewes	37	21.5	6.2	0.446**	±0.089
Romeldale rams	19	26.1	4.4	0.100	±0.153
Romeldale rams (m)	4	27.5	7.3	0.871	±0.081
Romeldale ewes	18	25.0	5.8	0.399	±0.134
<i>Central Experimental Farm, Ottawa</i>					
Shropshire ewes (m)	26	31.1	4.4	0.429*	±0.108
Leic. × Shrop. ewes (m)	25	33.9	5.2	0.226	±0.128
Romnelet ewes	10	28.3	4.0	-0.052	±0.224
Romnelet ewes (m)	28	29.1	4.8	0.003	—
Can. Corr. ewes	10	24.7	3.9	0.050	±0.213
Can. Corr. ewes (m)	16	27.8	5.9	-0.105	±0.167
Leic. × Oxford ewes (m)	8	34.7	6.3	0.257	±0.223
Suffolk ewes (m)	5	34.8	4.8	-0.417	±0.250
Cheviot × Leic. ewes (m)	5	40.8	5.4	-0.117	±0.298
<i>University of Saskatchewan, Saskatoon</i>					
Rambouillet rams	51	20.6	3.5	-0.116	±0.093
Rambouillet ewes	92	21.1	3.2	0.084	±0.104

¹ The breeds and crosses are shearlings with the exception of those indicated as mature (m).

* Significant at 5 per cent level.

** Significant at 1 per cent level.

The results of this analysis indicate that within breed groups the relationship of clean fleece weight to fibre thickness does not hold true in all cases. There are only five significant coefficients of correlation, all positive, out of 31 calculated. Two are significant at the 1 per cent level and three at the 5 per cent level of probability. The presence of 26 non-significant coefficients, including eight that are negative, indicates the lack of a definite relationship within a breed.

The data that have been presented are in agreement with those of other workers to the extent that, when a large number of fleeces of various grades and from various breeds are considered, there is a relationship between

clean fleece weight and fibre thickness. As the fibres become coarser, fleece weights tend to increase. However, the data further show that this relationship is not sufficiently close within breeds to be of any practical significance in a selection program for improving fleece weights by selecting on the basis of fibre fineness.

SUMMARY AND CONCLUSIONS

Individual fleece samples were collected, analysed for average clean fleece weight and fibre thickness, and the degree of relationship between these two characteristics determined.

In the preliminary analysis the clean fleece weights were assembled by grades and it was found that as the wool became coarser there was a definite trend towards increased clean weights. However, when the coefficients of correlation between clean weight and fibre thickness were calculated to determine the actual degree of relationship within a grade or breed it was found that only in a few cases was there any relationship between these two characteristics. It may be concluded that the relationship is sufficiently slight to not be evident within narrow ranges of fibre thickness.

ACKNOWLEDGMENTS

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BOOK REVIEW

"SCIENTIFIC HORTICULTURE". Published for the Horticultural Education Association by Jarrold & Sons Ltd., The Empire Press, Norwich, England.

After a lapse of some years, *Scientific Horticulture* has again made its appearance. Volume IX, 1949, contains a number of interesting articles.

An article on Soil Organic Matter and Composts is of particular interest to all horticulturists. The recommendations on the use of sewage sludge for soil fertility purposes should receive more attention in Canada, as we lose a considerable source of fertility, and the more general adoption of this system would aid in preventing the pollution of streams. The use of such sludge composted with straw would be of considerable value to small holders. Other articles deal with rapid tissue tests for mineral nutrients in plants and plant injection methods used by investigators in diagnosing nutritional deficiencies.

There is also an interesting account of modern applications of genetics and cytology to horticultural crops, dealing with mutations, bud sports and chimaeras, which help to explain apparent inconsistencies in the breeding behaviour of certain plants.

The article dealing with selective weed control is valuable for the historical data presented and summarizes the situation at the present time as regards the use of selective weedicides.

Another article of particular interest to horticulturists deals with preparation of composts for flats in greenhouses, etc.; rather definite recommendations are given for the preparation of a suitable compost.

— M. B. DAVIS
Dominion Horticulturist

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